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505. THE ACCURACY OF MEASUREMENTS OF THE MAMMARY GLANDS BY THE METHOD OF PALPATION

BY D. SHEPPARD

National Institute for Research in Dairying, University of Reading

(With 2 Figures)

It has recently been claimed by Swett (1) that it is possible to forecast the milk yield of a cow from measurements of its mammary glands made at 3–5 months old.

These measurements are, at present, made by the method of palpation, since the glands are sometimes difficult to locate, and are not easily accessible for measurement with an instrument.

The method used by Swett (2) to determine the dimensions of the glands is to press the finger and thumb lightly against the extreme lateral surfaces, when measuring the width of a quarter, or against the greatest extremity in front and behind, in the case of a length measurement of a half; the hand is then stiffened, and removed and placed against a ruler, the distance between the finger and thumb being then recorded.

Although this does not appear to be an ideal method for taking these measurements, it is a very simple one, and has the advantage that anyone should be able to carry it out, after suitable instruction, and with a little practice. Furthermore, provided that the person concerned can locate the gland successfully, it seems unlikely, at first sight, that any very large errors would be incurred in the taking of the actual measurement.

Swett himself reports the use of this method by a group of cow testers in Pennsylvania (1), on a group of calves selected to illustrate different degrees of gland development, and notes that there was very close agreement in the grading. Touchberry & Tabler (3) took a series of such measurements on ten calves at 3, 4 and 5 months of age; an analysis of variance was made for these data, and showed that there was a highly significant difference between calves, while the differences between judges was not significant on any occasion.

However, since agreement between judges does not necessarily indicate that their judgements are correct, it seemed worth while to test the method by getting a larger number of judges to use this method of measurement on a set of artificial 'glands', whose dimensions could be objectively measured.

EXPERIMENTAL METHOD

For this purpose a set of artificial 'glands' was made up of rubber tubing, blocked out with wood so as to be rectangular in section, and suspended within an 'udder' of thick hessian material. There were fifteen different 'glands', varying in length from 0·85 to 5·45 cm., and in width from 0·85 to 3·70 cm.

Each judge had to find the 'gland', press his finger and thumb lightly against it, then remove the hand, keeping these digits in the same position as far as possible, and measure the distance between them. The judges were allowed to do this as many times as they wished for each 'gland', but they were restricted to using the right hand throughout.

The subjects each did three complete tests, spaced out over a period of 6 months; a complete test consisting of two replicate 'measurements' on all fifteen 'glands', both for widths and lengths. Five subjects carried out these tests, of whom four were new to this type of task, while one had had considerable experience in using this method on calves.

RESULTS

Regression coefficients were calculated to examine the relationships between the estimated widths and lengths, and the objective measurements of the same sizes. The errors of these regressions were calculated, at a 0·05 level, so as to obtain a measure of the usefulness of the subjective assessments; and the various regression lines were compared one with another, so as to detect differences between judges, and differences for the same judge from one occasion to another.

These results are shown in Table 1.

RELATION BETWEEN TRUE SIZE AND ESTIMATED SIZE

When considering the value of this method of measurement, it is evidently essential to know the relation between the estimated sizes, and the true sizes, of the 'glands' being assessed.

For this purpose, regression equations and correlation coefficients were obtained for the relationship between the objective measurements and the subjective assessments of the same 'glands', for both widths and lengths, and for each of the three complete tests. These showed that for each subject there was a highly significant relationship between the true sizes and the estimates of these sizes. The level of significance being 0·001 in every case. The figures for these correlations are given in Table 1.

The regression equations which were obtained for each subject, for his estimates in all three tests, are also shown grouped together in the table, while the resulting regression lines for the five different subjects are shown in Figs. 1 and 2.

It can be seen that despite the high correlations which were found, the estimated sizes are generally smaller than the true sizes, and are significantly smaller with the exception of subject E's estimates of widths. It should be also noted that the estimated sizes given by the expert (subject A) were no nearer to the true sizes than were those of the unskilled subjects.

This evidently limits the value of this method of measurement, but it might still be justifiable to compare directly glands which have been assessed as being of the same size by one judge, although it might be misleading to do so when the assessments had been made by different judges.

A word of warning is necessary when examining the data shown in Table 1. As will be seen, a method of analysis of variance was used which enables one not only to compare the slope of any two regression lines, but also their intercepts on the y-axis (4), where y is the dependent variable; in this case the estimated size.

Such an intercept is of course obtained from an extrapolation of the calculated regression line since the true sizes were never less than 0·85 cm. An extrapolation may be justified since, over the range of sizes used, the relation between the true and estimated sizes appeared to be a linear one. This would mean, however, that as the true sizes become progressively smaller, certain subjects would be led to give sizes which would decrease to zero, and finally become negative.

In fact, subsidiary experiments suggest that if the distance between the fingers could be automatically registered, negative sizes would result in certain cases, after allowance is made for the thickness of the skin. Needless to say, however, no subject will give a size which, after correction, he knows will be zero or negative, and on such occasions he therefore tries again until he gets something more reasonable.

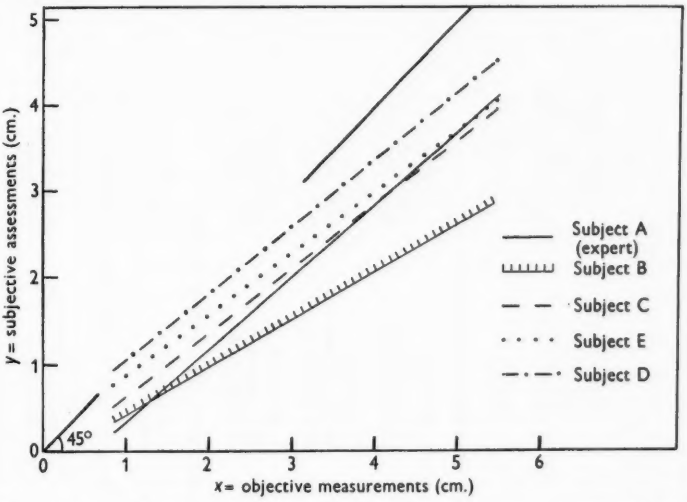

Fig. 1. Regression lines of subjective assessments of lengths on objective measurements of lengths.

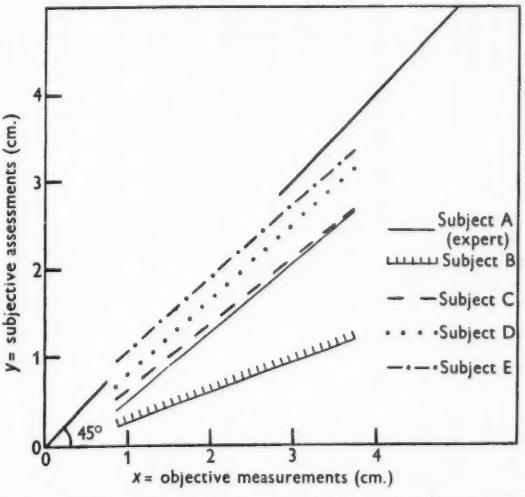

Fig. 2. Regression lines of subjective assessments of widths on objective measurements of widths.

Thus it seems that though it may be justifiable to consider the difference in intercepts when examining the more physiological mechanisms involved in making these assessments, in practice the curve would not, on extrapolation, remain linear. For this reason, therefore, it is felt that too much value should not be attached to the significance levels given for these differences.

Table 1. *Relation between subjective assessments and objective measurements*

Subjects	Widths A	B	C	D	E
Correlations between true and estimated sizes (with significance levels in brackets)	1. +0·97 (0·001) 2. +0·97 (0·001) 3. +0·97 (0·001)	1. +0·80 (0·001) 2. +0·85 (0·001) 3. +0·80 (0·001)	1. +0·89 (0·001) 2. +0·94 (0·001) 3. +0·91 (0·001)	1. +0·94 (0·001) 2. +0·86 (0·001) 3. +0·86 (0·001)	1. +0·77 (0·001) 2. +0·87 (0·001) 3. +0·94 (0·001)
Regression equations for estimated sizes (y), on true sizes (x)	$y=0{\cdot}80x-0{\cdot}32$	$y=0{\cdot}54x-0{\cdot}08$	$y=0{\cdot}76x-0{\cdot}13$	$y=0{\cdot}85x+0{\cdot}03$	$y=0{\cdot}84x+0{\cdot}27$
Error of regression lines (cm.)	∓0·44	∓1·18	∓0·78	∓1·12	∓1·10
Analysis of variance between tests. On different days.					
	Tests 1–2	Tests 1–2	Tests 1–2	Tests 1–2	Tests 1–2
Difference in intercepts	0·001 level	0·001 level	Not significant	0·20 level	Not significant
Difference between regression lines	0·05 level	Not significant	Not significant	Not significant	Not significant
	Tests 1–3	Tests 1–3	Tests 1–3	Tests 1–3	Tests 1–3
Difference in intercepts	0·01 level	Not significant	Not significant	0·001 level	0·05 level
Difference between regression lines	Not significant	Not significant	Not significant	0·05 level	Not significant
	Tests 2–3	Tests 2–3	Tests 2–3	Tests 2–3	Tests 2–3
Difference in intercepts	0·001 level	Not significant	Not significant	0·01 level	Not significant
Difference between regression lines	Not significant	Not significant	Not significant	Not significant	Not significant
Analysis of variance between first test ('A') and second test ('B') for all days grouped.					
Difference in intercepts	Not significant	0·05 level	Not significant	0·05 level	0·05 level
Difference between regression lines	Not significant	Not significant	0·05 level	0·05 level	0·20 level
Analysis of variance between true sizes (T.S.) *versus* estimated sizes (E.S.) for all days grouped.					
	T.S. > E.S.	T.S. > E.S.	T.S. > E.S.	T.S. > E.S.	T.S. ≒ E.S.
Difference in intercepts	0·001 level	0·001 level	0·001 level	0·010 level	0·05 level
Difference between regression lines	0·001 level	0·001 level	0·001 level	0·05 level	0·20 level

Tests 1–2–3 = tests on different days. Tests A–B = repeat tests, same day.

Subjects	Lengths A	B	C	D	E
Correlations between true and estimated sizes (with significance levels in brackets)	1. +0·91 (0·001) 2. +0·97 (0·001) 3. +0·97 (0·001)	1. +0·79 (0·001) 2. +0·96 (0·001) 3. +0·91 (0·001)	1. +0·95 (0·001) 2. +0·98 (0·001) 3. +0·97 (0·001)	1. +0·90 (0·001) 2. +0·96 (0·001) 3. +0·91 (0·001)	1. +0·90 (0·001) 2. +0·95 (0·001) 3. +0·87 (0·001)
Regression equations for estimated sizes (y), on true sizes (x)	$y=0{\cdot}84x-0{\cdot}53$	$y=0{\cdot}55x-0{\cdot}15$	$y=0{\cdot}74x-0{\cdot}11$	$y=0{\cdot}71x+0{\cdot}16$	$y=0{\cdot}76x+0{\cdot}31$
Error of regression lines (cm.)	∓1·14	∓1·43	∓1·02	∓1·35	∓1·57
Analysis of variance between tests. On different days.					
	Tests 1–2	Tests 1–2	Tests 1–2	Tests 1–2	Tests 1–2
Difference in intercepts	0·001 level	0·20 level	0·05 level	0·20 level	Not significant
Difference between regression lines	0·001 level	Not significant	Not significant	Not significant	Not significant
	Tests 1–3	Tests 1–3	Tests 1–3	Tests 1–3	Tests 1–3
Difference in intercepts	0·001 level	0·01 level	0·001 level	Not significant	0·20 level
Difference between regression lines	0·001 level	Not significant	Not significant	Not significant	Not significant
	Tests 2–3	Tests 2–3	Tests 2–3	Tests 2–3	Tests 2–3
Difference in intercepts	Not significant	Not significant	0·20 level	Not significant	Not significant
Difference between regression lines	Not significant	Not significant	Not significant	Not significant	Not significant
Analysis of variance between first test ('A') and second test ('B') for all days grouped.					
Difference in intercepts	Not significant	0·20 level	0·001 level	0·20 level	Not significant
Difference between regression lines	Not significant	Not significant	0·20 level	Not significant	Not significant
Analysis of variance between true sizes (T.S.) *versus* estimated sizes (E.S.) for all days grouped.					
	T.S. > E.S.	T.S. > E.S.	T.S. > E.S.	T.S. > E.S.	T.S. > E.S.
Difference in intercepts	0·001 level	0·001 level	0·001 level	0·001 level	0·001 level
Difference between regression lines	0·001 level	0·001 level	0·001 level	0·001 level	0·001 level

Tests 1–2–3 = tests on different days. Tests A–B = repeat tests, same day.

It should perhaps be pointed out that, over the range of sizes used here, which were chosen as being representative of 'real' glands, the subjects had no occasion to feel doubtful of the measurements they were giving.

AMOUNT OF ERROR IN THESE ESTIMATES

Although the estimated sizes are significantly different from the true sizes, it remains possible that from a regression equation for estimated sizes on true sizes, using an apparatus and method such as that described in this paper, it would be possible to convert the estimated sizes into true sizes, and even to compare one judge with another.

The usefulness of such a procedure would depend upon the closeness of the relation between the estimated sizes and the true sizes; in fact, the error about the regression line.

The error of this line at a 0·05 level was therefore calculated for each subject, and these are shown in Table 1, expressed in cm. It can be seen that these errors are large in comparison to the range of sizes used in the experiment (this being 4·60 cm. for length and 2·85 cm. for width). It was of interest to note that the error for the expert's estimates of width was less than that of any of the other four subjects, although one of the untrained subjects had a smaller error for assessing lengths.

The fact that this error is so large is another serious drawback to this method, particularly since the error is likely to be larger when assessing the glands of calves. where the glands may be covered with layers of fat and may be hard to locate. In addition, a calf cannot readily be held still while these assessments are being made.

CHANGES IN THE RELATION BETWEEN TRUE SIZES, AND ESTIMATED SIZES

Since this method of assessing sizes has already been much used, and will probably continue to be used until some better method can be found to replace it, it is of importance to know whether the relationship between the estimated sizes and the true sizes would remain the same over a period of time.

For this purpose, the estimated sizes in the first, second, and third tests for each judge, were compared by the analysis of variance method, so that any changes in these estimated sizes with time could be detected. The results of this analysis are shown in Table 1.

When considering these results, it should be remembered that the expert (subject A) had not only had considerable practice in assessing the sizes of mammary glands in calves before carrying out her first test on these artificial 'glands', but that, in addition, she continued making these measurements in the intervals between her three tests. The non-expert subjects had had no practice, either with real or artificial glands, before or during the period over which their three tests were carried out.

In most cases, it seemed as if there was a fairly constant relationship between the true sizes and the estimated sizes for each subject, and that this relationship did not vary significantly from one occasion to another. There were exceptions, however: thus the estimated widths for subject D became progressively smaller from the first to the third tests, although only those in the first and last tests were significantly different. Subject E showed a similar trend in her estimates of lengths, but for her none of the regression lines were significantly different.

There were also significant differences between the expert's first and second, and first and third tests for lengths, and between her first and second tests for widths. Her estimates for lengths were nearer to the true sizes, on the second and third occasions,

than they were in the first; while for the widths, on the second occasion her estimates were more like the true sizes than they were on the first occasion, although on the third occasion they became more similar to those given in the first test.

These changes, however, are less discouraging than they seem, since this subject was informed at the end of her first test that she tended to reduce the true sizes when assessing them. She had not previously suspected this, and although she tried not to allow this knowledge to affect her technique, it evidently did do so, so that she did not reduce the true sizes by so much when re-tested. The fact that in the third test her estimated widths were midway between those for her first and second tests, suggested that she was returning to her more natural procedure.

These effects, occurring with the expert subject, are not surprising, and are in accord with the findings on the effects of knowledge of results on performance made in recent years by psychologists. The fact that such changes did occur does not necessarily indicate that her estimates would normally have altered over a period of time, nor need it necessarily be assumed from the changes occurring with certain of the non-expert subjects, that self-consistency is not possible; since, had they been given the opportunity for a considerable amount of practice, they might have attained a stable level at which the relation between true and estimated sizes would have remained reasonably constant.

To obtain further information on such changes, analyses of variance were made so as to compare the repeat trials made on each day by each subject. The results for each day were compared separately for the expert, but for the non-experts the results for each day were grouped together, so that the first tests (tests A) for all 3 days, were compared to the second tests (tests B) for all 3 days.

The expert did not change significantly between her A and B series, on any one of the 3 days, nor on all the 3 days grouped either for widths or lengths. Neither were the differences between A's and B's significant for the non-expert subjects, with the exception of subject D, for whom the estimated widths of tests A were significantly greater than the estimated widths of tests B. Thus the same trend was evident for subject D during tests, as had been noted between tests, which was a significant trend to 'shrink' the true sizes more with time.

Since subject D knew that the expert had reduced the true sizes before he began his own tests, it is just possible that he might have tried to avoid such a tendency at first, and at the beginning of each test, but that he reverted to a more natural procedure as time went on. Nevertheless, it remains possible that there was a real change in his natural procedure with time.

Once again, however, should it prove necessary to continue with this method of assessing gland sizes, an apparatus such as that described here could be used to detect such changes, and an allowance could then be made for these when dealing with the results.

DIFFERENCES BETWEEN JUDGEMENTS OF LENGTHS AND WIDTHS

It is evident that the error for assessments of lengths is greater than that for the estimates of the widths, but since this could have been merely a result of the errors being proportional to the sizes measured, when, as in this case, the average length was considerably greater than the average of the widths, the scatter of the estimated sizes on certain identical lengths and widths was examined.

It did seem that the expert (subject A), was less consistent in her judgements of

lengths than in those for widths, while subject D appeared to be somewhat more self-consistent in judging lengths. The differences were not great, however, and the other three judges appeared to be equally self-consistent by either method.

It seems, therefore, that the slight differences in technique necessary for assessing these two different measurements (such as the position of the hand) do not make any great differences to the accuracy of the measurements, accuracy being considered in terms of self-consistency.

THEORIES TO ACCOUNT FOR DIFFERENCES BETWEEN JUDGES

Since the evidence obtained from these experiments is sufficient to demonstrate clearly that there may be large differences between judges and, further, that the estimated sizes may be very different to the true sizes, it is of interest to try to find out what it is that brings about these differences.

Swett, although he claims that there is often considerable agreement among subjects, has also noted that certain differences may occur. Thus, in a letter circulated to co-operators in his experiment(5) he notes that he had received some unusually large measurements of width from one or two of his co-operators, and was uncertain whether to attribute this to unusual techniques in taking the measurements, or to unusual shapes of the glands which had been measured. Since the evidence obtained from this experiment suggests that it is unusual for anyone to exaggerate the sizes of the glands, it seems likely that Swett also shrinks the 'true' sizes, and, from his own evidence, that he does this to a greater extent than other people.

Swett also notes in his letter that judges should not exert any pressure when measuring lengths, or at least only enough to give the impression of a 'snug fit'. It would seem therefore that Swett has noticed that certain judges give sizes which seem to him to be too small, and considers that this could be due to exerting pressure on the gland, and therefore bringing the fingers too close together at the outset.

It is conceivable that this could be a factor of some importance when measuring the glands of a calf, and it was possible to show that the average pressure used in the tests described here, by a group of fourteen subjects, was equivalent to a static load of as much as 4·0 lb. The standard deviation for these pressures was 3·7 lb., the distribution being positively skewed.

Nevertheless, observations made in the experiments described here suggest that errors, which result from using too much pressure, are not of sufficient importance to account for the large discrepancies that can occur between the true and estimated sizes. For one thing, the 'glands' used in these experiments are incompressible, and for another, it is possible to observe the fingers drawing together during the interval between the sizing of the gland and the placing of the fingers against the ruler.

What seems to happen, then, is that the subject, in tensing his muscles so as to hold his fingers rigidly in one position, in fact tends to contract these muscles, and so draws the fingers closer together.

The other possible explanation for the reducing of the sizes, namely the recovery of deformed skin tissue at the finger-ends in the interval between the sizing of the glands and the measurement of the distance between the fingers, can be ruled out as a result of certain experiments carried out by the author in connexion with another problem. These

indicated that the recovery of the skin tissue after compression is too slow to make such large differences as occur here between the estimated and the true sizes.

Furthermore, this would only account for a constant difference on any size, whereas, in these experiments, the larger the 'gland', the more the estimated size differs from the true size.

Since it seemed that the most likely assumption was that the alteration in sizes occurred as a result of the muscles contracting when the fingers were tensed, a few simple experiments were carried out in an attempt to test this theory.

DEVELOPMENTS OF THE MUSCLE CONTRACTION THEORY

The first, and simplest of these experiments, was to see if it was possible to produce a continual reduction of an original measurement. This was done by the simple method of asking the subjects to place their fingers at a fixed distance apart against a ruler, remove the fingers for a few seconds attempting to 'hold' this measurement, and then return them to the ruler. They then attempted to hold their new measurement, and so on. It was found that four out of the five subjects originally used, including the expert, gave a series of continually decreasing sizes.

This seemed to confirm the muscular contraction theory, but it seemed worth while to examine the effect of time on estimates of these sizes. If these alterations are a result of the distance between the fingers continually contracting, it seemed likely that extending the time between the taking of a measurement, and the placing of the fingers against a ruler, might tend to make the subject give smaller sizes than those usually given by him.

This was tried with five subjects, including four of those originally used, but only two gave estimated sizes for the longer time interval which were smaller than those given with the more normal time intervals, and the differences were not significant in either case. It seemed likely, however, that these differences would have become significant if more tests had been carried out.

Since this experiment yielded few positive results, it is likely that the original assumption was too simple, and in fact, it seems probable that a task of this kind is of the familiar 'cybernetic' nature frequently noted in similar psycho-physiological tasks. If this is the case, what probably happens with most subjects is that the distance between the fingers contracts by a certain amount, this is then 'noticed', and some kind of 'feed-back mechanism' is set in operation to affect the extensor muscles, and the contraction ceases, or nearly so.

If this is correct, it is interesting that this 'mechanism' should operate so that the true sizes are generally decreased to what is more like a constant ratio of their original size, rather than by a constant amount, although this would seem to comply with the operation of the Weber-Fechner law.

One further experiment was carried out in connexion with this muscular contraction theory. It seemed just possible that if the reduction in sizes was due to muscular contraction, a person with strong fingers might tend to give smaller estimated sizes, than would one with relatively weak fingers.

It proved possible to measure the strength of grip with a finger dynamometer, and a correlation was obtained between the maximum grips recorded by each of sixteen subjects (including the five originally used), and their estimates of the median length

and width used in this experiment. This estimate was obtained from a regression equation of the type described previously.

The correlation between these two sets of measurements was not high, being 0·52 for lengths (significance level 0·05), and 0·47 for widths (significance level 0·10). Nevertheless, this does indicate some relationship, and it seems likely that some of the differences in the estimated sizes could be attributed to differences in the finger strength of the subjects carrying out these tests.

The relationship was such that the person with strong fingers tended to reduce the sizes more than did the person with weak fingers.

It is possible to obtain some idea of the possible variations in this method of measurement from the estimates given by these sixteen subjects. Thus for a true width of 3·08 cm., including 'skin size', the standard deviation of the estimates was 0·81 cm., with a mean size of 2·89 cm. For a true length of 3·95 cm., the standard deviation of the estimates was 0·72 cm., and the mean estimate was 3·51 cm. In each case only five subjects gave sizes which were equal to, or larger than, the true size.

CONCLUSION

When gland sizes are assessed by the method of palpation, the results would seem to depend to some extent on the finger strength of the judge as well as on the true sizes of the objects being measured.

For this reason, therefore, and taking into account the possible effects of the time taken to make the measurement, the changes which may occur in the relation between the estimates and the true sizes, and the general inaccuracy of the method, it seems necessary to use some better method of assessing the gland sizes in the calf.

However, should this not prove to be possible, or immediately possible, an apparatus such as that described in this paper could be used to give some indication of the accuracy of such assessments for any judge, and also to show the relation between his estimates and the real sizes. Thus it would be possible to obtain a best estimate of the real size of a mammary gland from a subjective assessment of this size, and, if desired, to compare one judge with another with some degree of confidence.

SUMMARY

1. An investigation was carried out, using objectively measurable stimuli, to examine the method of assessing the size of the mammary glands of calves by palpation. The method was that described by Swett.

2. The results indicate that the estimated sizes may be very different from the true sizes, although the two are generally highly correlated. In most cases the estimated sizes are significantly smaller than the true sizes.

3. Different judges give very different estimated sizes for the same true sizes, thus it is not justifiable to compare directly glands which have been stated to be of the same size by different judges.

4. Despite the high correlation between the true sizes and the estimated sizes, there is a considerable error involved (at a 0·05 level) in attempting to predict one from the other, in comparison to the range of sizes which was used. This is true even for a judge experienced in the use of this method.

5. Attempts to examine the consistency of the relationship between estimated and true sizes, over a period of 6 months, showed that this relation changed for certain subjects. Only one subject showed any such change during tests made on one day. Such changes as there were may have been a consequence of the subjects having been informed of their results.

6. Only two subjects showed any differences in accuracy between their judgements on widths or lengths when these were of comparable sizes. These differences were not great.

7. The fact that the estimated sizes are usually smaller than the true sizes seems likely to result from the muscles of the fingers contracting when the judge is tensing his fingers in an attempt to hold them rigid.

8. Subsidiary experiments showed that the estimated sizes became smaller than usual if the time taken to make the measurement was increased, for certain of these subjects.

9. Subjects who gave the smallest estimated sizes appeared to be those who could exert the greatest strength of grip with the operative fingers, while those who were relatively weak in the fingers tended to exaggerate the sizes. The strength of grip was measured with a finger dynamometer.

The author wishes to acknowledge his gratitude to Miss G. Elliot, of Reading University, for introducing this problem to him, and also for acting as a subject for the experiment. He also wishes to thank Mr F. A. Glover for his advice on the construction of the test material; Miss S. Carrinci for invaluable assistance, both in carrying out the experiments, and in analysing the data; his subjects for performing the tests for him, and the Agricultural Research Council for a grant.

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637.124.2

506. THE IMPORTANCE OF MACHINE MILKING RATE IN DAIRY COW MANAGEMENT AND BREEDING

By F. H. DODD and A. S. FOOT

National Institute for Research in Dairying, University of Reading

(With 2 Figures)

The discoveries of the last decade have considerably improved our understanding of the neural and hormonal mechanisms controlling milk ejection. This, in turn, has stimulated research into the many practical problems concerned with hand and machine milking. The discovery that milk ejection is a transitory mechanism encouraged the study of the rate of machine milking of dairy cows since it was inferred that less milk is obtained when milking is slow. This research work has shown that there are very wide differences in the machine milking rate of cows in the same herd, even when they are under identical management (1, 2, 3). However, the milking rate of a particular cow is relatively stable over long periods (2, 3, 4) and cannot be altered even by severe changes in management (5, 6, 7). The explanation of this appears to be that milking rate is mainly controlled by the structure of the teat orifice (8).

The results given in this paper carry this study a stage further. They show the effect of inherent milking rate on milk yield, milk composition and the rate of decline of milk yield. In addition, some preliminary observations on the inheritance of milking rate are given.

SOURCE OF DATA

The measurements of milking rate, milk yield and milk composition used in this analysis were all made in the herd of the National Institute for Research in Dairying. For details of the method of collection of the milking-rate data reference should be made to another publication (3). The lactation yields of the cows were all compiled from the daily weighing of the milk produced by each cow at each milking. Samples of milk for milk-composition analysis were collected on one day each month. Elsewhere, Bailey (9) has described in some detail the management, feeding and breeding of the herd.

RESULTS

For convenience the following terms have been used in presenting the results:

'Peak flow' (l6/min)—maximum milking rate attained in any minute during milking.

'Standard lactation yield' (lb.)—amount of milk produced in 305 days or until dry, whichever is the shorter period.

'Lactation fat percentage'—mean weighted fat percentage in the total lactation period.

'Lactation solids-not-fat percentage'—mean solids-not-fat percentage in the total lactation period.

'Lactation length' (days)—days in milk from 4 days after calving until dry.

'Persistency' (days)—the number of days after calving before the weekly yield falls below one-half of the maximum weekly production.

The milking-rate records used in the calculation of the regression coefficients with milk yield, milk composition, maximum daily yield, lactation length and persistency were all collected from eighty-six animals in about the fifth week of their first lactations. Only heifer results were used in order to eliminate the effect of lactation age and also to avoid the effects of other experiments carried out on older cows. The mean values of the various measures used in the analyses are given in Table 1, together with their standard deviations and coefficients of variation. The values of the coefficients of variation for lactation

Table 1. *The means, standard deviations and coefficients of variation of the various measures of milk yield, milk composition, persistency and milking rate used in the analysis*

	Mean and standard error	Coefficient of variation
Standard lactation yield (lb.)	6768±1633	24·13
Lactation fat (%)	3·943±0·368	9·33
Lactation solids-not-fat (%)	9·339±0·220	2·36
Lactation length (days)	297·3±52·62	17·70
Maximum daily yield (lb.)	34·22±5·722	16·72
Persistency (days)	228·6±63·67	27·85
Peak flow (lb./min.)	4·040±1·417	35·07

milk yield, fat percentage and solids-not-fat percentage are very similar to those found by other workers (9, 10). It is interesting to observe that the coefficient of variation for peak flow is 35%, a much greater value than that of any of the other measures used.

In the analyses given below two methods of estimating the relationship between factors has been used. Linear regression coefficients and partial regression coefficients were calculated, the former give the regression between pairs of variates whilst the latter give their regression after correction for one or more interacting factors.

THE RELATIONSHIP BETWEEN MILKING RATE (PEAK FLOW) AND MILK YIELD AND COMPOSITION

In Table 2 the regression coefficients of peak flow on (*a*) standard lactation yield, (*b*) lactation fat percentage, and (*c*) lactation solids-not-fat percentage are presented. These show that for each pound per minute increase in peak flow the standard lactation

Table 2. *The relationship between milking rate and milk yield, milk composition and persistency*

x	*y*	Regression coefficient (*byx*)
Peak flow (lb./min.)	Standard lactation yield (lb.)	+418·86±117·2**
	Lactation fat (%)	−0·0348±0·0281
	Lactation solids-not-fat (%)	−0·0411±0·0162*
	Lactation length (days)	+7·920±3·950*
	Maximum daily yield (lb.)	+0·973±0·426*
	Persistency (days)	+10·647±4·762*

* Significant at the 5% level of significance.
** Significant at the 1% level of significance.

yield increases by 419 lb., the lactation fat percentage decreases by 0·035 and the lactation solids-not-fat percentage by 0·041. The relationship with yield is significant at the 1% level of significance, with solids-not-fat percentage at the 5% level, whilst the regression coefficient on fat percentage is not significant.

The partial regression coefficients given in Table 3 show that the regression of peak flow on lactation milk yield corrected for solids-not-fat percentage and fat percentage is

highly significant and similar in magnitude to the simple regression coefficient. Neither of the coefficients of peak flow on lactation solids-not-fat percentage and fat percentage is significant.

Table 3. *Partial regression coefficients showing the relationship between milking rate and milk yield and milk composition*

y	X_1	X_2	X_3	Partial regression coefficient
Standard lactation yield (1000 lb.)	Peak flow (lb./min.)	Lactation fat (%)	Lactation solids-not-fat (%)	$byx_{1\cdot23} = +0\cdot3838 \pm 0\cdot1203^{**}$ $byx_{2\cdot13} = +0\cdot1178 \pm 0\cdot5040^{*}$ $byx_{3\cdot12} = -0\cdot9555 \pm 0\cdot7820$
Lactation solids-not-fat (%)	Peak flow (lb./min.)	Lactation fat (%)	Standard lactation yield (1000 lb.)	$byx_{1\cdot23} = -0\cdot0236 \pm 0\cdot0152$ $byx_{2\cdot13} = +0\cdot2806 \pm 0\cdot0549^{**}$ $byx_{3\cdot12} = -0\cdot0185 \pm 0\cdot0131$
Lactation fat (%)	Peak flow (lb./min.)	Lactation solids-not-fat (%)	Standard lactation yield (1000 lb.	$byx_{1\cdot23} = -0\cdot0036 \pm 0\cdot0270$ $byx_{2\cdot13} = +0\cdot8539 \pm 0\cdot1669^{**}$ $byx_{3\cdot12} = +0\cdot0091 \pm 0\cdot0232$

* See footnote to Table 2.

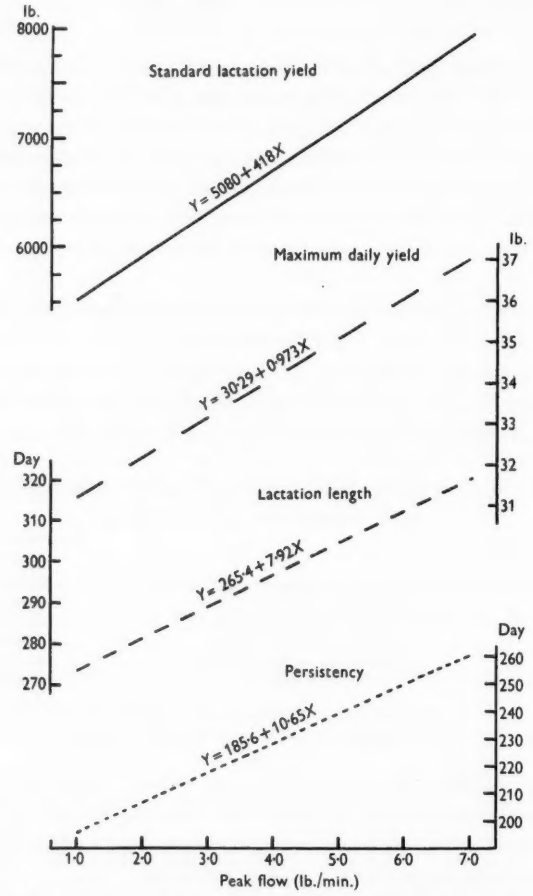

Fig. 1. The relationship between a cow's inherent milking rate (peak flow) and standard lactation yield, maximum daily yield, lactation length and persistency.

Milking rate and its relationship with maximum daily yields, lactation length and persistency

The regression coefficients in Table 2 show that peak flow is related to maximum daily yield, lactation length and persistency. With each pound per minute increase in peak flow rate there is a mean increase of 0·9 lb. in the maximum daily yield, 7·9 days in lactation length and 10·6 days in the persistency index; all these coefficients being significant at the 5% level of significance. The regression lines of these three factors with peak flow rate are given in Fig. 1, together with the similar regression line between standard lactation yield and milking rate.

In Table 4 the partial regression coefficients of these three factors and peak flow on standard lactation yield are given. All the partial coefficients are positive and highly significant, except that of peak flow which is positive and not significant. This means that the highly significant regression between peak flow and standard lactation yield shown in Tables 2 and 3 is due to interaction and that this relationship exists only because peak flow affects maximum yield, lactation length and persistency.

Table 4. *Partial regression coefficients and* b*-primes calculated from data on milking rate and standard lactation yield, maximum daily yield, lactation length and persistency*

Y	X_1	X_2	X_3	X_4	Partial regression coefficients
Standard lactation yield (1000 lb.)	Peak flow (lb./min.)	Maximum daily yield (lb.)	Lactation length (days)	Persistency index (days)	$byx_{1\cdot234} = +0\cdot0671 \pm 0\cdot0837$ $byx_{2\cdot134} = +0\cdot0684 \pm 0\cdot0222$** $byx_{3\cdot124} = +0\cdot8917 \pm 0\cdot2512$** $byx_{4\cdot123} = +1\cdot1488 \pm 0\cdot1955$** $b'yx_{1\cdot234} = 0\cdot0582$ $b'yx_{2\cdot134} = 0\cdot2397$ $b'yx_{3\cdot124} = 0\cdot3137$ $b'yx_{4\cdot123} = 0\cdot4479$

* See footnote to Table 2.

An additional interesting result is obtained by calculating the b-primes or standard partial regression coefficients from these data. The relationship of these b-primes indicates the relative influence of maximum daily yield, lactation length and persistency on standard lactation yield. They are given in the same table and indicate that in this group of cows the most important factor influencing standard lactation yield was persistency, followed by maximum daily yield and then by lactation length.

Milking rate (i.e. peak flow) and age at culling

The management of the herd in which this study was carried out has been outlined in other papers (3, 9). The herd was self-contained, apart from a Friesian section, and since an effort was being made to increase herd size culling was not severe. Culled animals were mainly low yielding, infertile or badly infected with mastitis, and as far as could be discovered no regard was paid to milking rate. Nevertheless, since it was possible that there was an involuntary culling of the slower milking cows this aspect was investigated. During the years in which these records were collected, sixty-eight cows were culled. Since many of these animals had their heifer lactation before the start of this study it has been necessary to use the earliest lactation rate of milking record available. The regression of lactation age at culling lactations on peak flow (lb./min.) is $b = +0\cdot5830 \pm 0\cdot101$, a highly significant coefficient of considerable size. This means that cows with a maximum milking rate of 8 lb./min. tend to remain in the herd about three

lactations longer than cows with rates of 3 lb./min. The partial regression coefficients of peak flow and standard lactation yield on age at culling are given in Table 5, both these positive coefficients are significant.

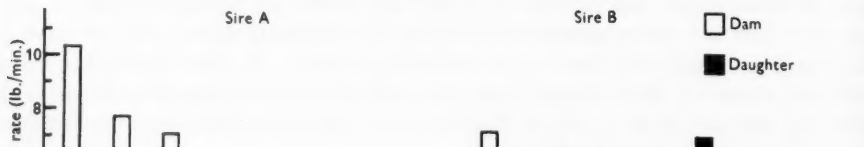

Fig. 2. Dam-daughter comparisons of maximum milking rate from two series of matings.

PRELIMINARY OBSERVATIONS ON THE INHERITANCE OF MILKING RATE

At the present time there are insufficient data for a detailed investigation of the inheritance of milking rate, particularly since artificial breeding has resulted in the births of daughters

Table 5. *The regression coefficient of lactation age at culling on peak flow and the partial regression coefficients when correction is made for standard lactation yield*

Y	X_1	X_2	Regression coefficients
Age at culling (lactations)	Peak flow (lb./min.)	Standard lactation yield (1000 lb.)	$byx_1 = +0{\cdot}5830 \pm 0{\cdot}101$**
			$byx_{1.2} = +0{\cdot}5108 \pm 0{\cdot}103$**
			$byx_{1.2} = +0{\cdot}2226 \pm 0{\cdot}109$*

* See footnote to Table 2.

of more than twelve bulls during this period. Unfortunately, it was not possible to restrict this particular analysis to the records of first lactation animals since these results were not available for many of the dams. Since the effect of age on milking rate is comparatively small (3) this should not affect the validity of the results.

Where first lactation milking-rate records were not available for both dam and daughter the records used were selected in the following way. If both had records for another lactation age (i.e. second or third, etc.) then these were used; if not the two records showing the least difference in age were used. A similar system was adopted for the calculation of coefficients for the sibs and half-sibs.

The simple measure of inheritance used was to calculate the correlation coefficients between the peak-flow records of dams and daughters and sibs and half-sibs. The correlation coefficients of the milking rates (peak flow) of 40 dam-daughter pairs and 35 pairs of sibs and half-sibs are 0·3720 and 0·3746 respectively. Both these coefficients are significant at the 5% level of significance.

The striking influence of the sire on these relationships is illustrated in Fig. 2 where the peak-flow rates of dam-daughter pairs of two bulls are presented. In each case all the pairs so far available have been included. While the milking rates of the daughters of bull B are similar to their dams, those of bull A are in all cases very much slower. Five of the eight daughters of this bull had maximum milking rates of about 2 lb./min., and

as a result the mean duration of milking of each cow was over 15 min. In addition, two daughters of this bull were culled within the first month after calving because of extreme slowness of milking.

DISCUSSION

From the results reported above it is apparent that the milk yield of a cow is related to her inherent milking rate. This increase in milk yield with unit increase in milking rate is not only significant at the 1% level but of considerable size. With each increase of 1 lb./min. in peak flow there is a mean increase of approximately 400 lb. in the standard lactation yield. Similarly, Table 2 shows that there is a relationship between milking rate and maximum daily yield, lactation length and persistency. The partial regression coefficient of peak flow on standard lactation yield corrected for maximum daily yield, lactation length and persistency (Table 4) is not significant. It is clear, therefore, that it is through these three factors that milking rate affects standard lactation yield.

Although it is impossible to say with certainty which is the dependent factor in this relationship it is reasonable to suppose that lactation milk yield is dependent on milking rate. On the one hand there appears to be no reason why persistency and lactation length should affect milking rate, and on the other hand it is known that the anatomy of the teat sphincter has a far greater effect on milking rate than has daily yield (8).

The effect on lactation yield can be explained by the accepted theory that since milk ejection is only a temporary phenomenon slow milking cows have larger amounts of residual milk left in the alveolar tissue at the end of each milking. This depresses the rate of secretion and reduces persistency, and as a result decreases lactation yields. Another possible explanation is that impatient milkers remove the milking machines from slow-milking cows before milk flow has ceased, and as a result milk yields are reduced. Although the latter cannot be ignored, the former explanation seems to be the more probable.

The data in Tables 2 and 3 reveal that milking rate has little effect on milk composition. Neither of the regression coefficients of peak flow on lactation fat percentage is significant. Although the simple regression of peak flow on lactation solids-not-fat percentage is negative and significant at the 5% level of significance, this appears to be due to interaction since the partial regression of peak flow on solids-not-fat percentage corrected for standard lactation yield is not significant (see Table 3).

Although the persons responsible for culling in the herd did not have access to milking-rate records, slow-milking cows were culled more quickly than fast milkers. The partial regression coefficients (Table 5) indicate that culling of slow milkers was not entirely due to their lower milk production since the regression of milking rate on culling age, independent of yield, is significant.

The preliminary data on the inheritance of milking rate confirm the findings of Beck *et al.* (2) that milking rate is an inherited character. The significant correlation between milking rates of sibs and of dams and daughters indicates that it is through breeding and selection that cows of improved milking rate can be obtained. An illustration is given in Fig. 2, where the adverse effect of a bull on the milking rates of his daughters is strikingly shown. This is an interesting demonstration of how an inherited anatomical factor, namely the structure of the teat orifice, can mask the inherent milk-producing ability of an animal. A cow may have an excellent genotype for the rapid secretion of milk yet be unable to express this ability because slow milking prevents the milker from

removing the milk from the udder. In addition, it has been shown by Lush (11) that susceptibility to mastitis is inherited. Since it has also been demonstrated that milking rate and susceptibility to mastitis are closely related (12), it is possible that the inheritance of susceptibility to mastitis is a reflexion of the inheritance of machine milking rate.

The results of research work carried out on machine milking rate in recent years can be summarized in the following way. Although milking rate is known to influence milk yields the application of this knowledge to farm practice has presented difficulties. It has been found that the machine milking rates of individual cows are inflexible, and up to the present time it has not been possible to affect changes in this rate by altering herd management. However, the milking rates of individual cows are very different, the differences being sufficient to affect their milk yields to a considerable extent. Since these milking rates are inherited, increased milk yields could be obtained by breeding for faster milking. In this connexion it must be realized that faster milkers require more careful management as they are more susceptible to mastitis (12).

This analysis has limitations since the data were all collected in one herd of cows. Further evidence is being collected in other herds, and will be presented at a later date.

SUMMARY

An analysis has been carried out on the milk yield, milk composition and milking-rate records collected in one herd of dairy cows.

It has been shown from the results of eighty-six heifers that for each pound per minute increase in inherent milking rate (i.e. peak flow) the standard lactation yield increases by 419 lb., the maximum daily yield by 0·9 lb., the lactation length by 8 days and the persistency index by 10 days, whilst the lactation solids-not-fat percentage decreases by 0·03 and the fat percentage by 0·04. All except the last coefficient were significant, although it was also shown that the relationship with solids-not-fat percentage was due to an interaction. Similarly, by the calculation of partial regression coefficients it was demonstrated that the relationship between milking rate and standard lactation yield was due to the effect of milking rate on maximum daily yield, lactation length and persistency.

In the herd from which the records were obtained slow-milking cows were culled more quickly than fast milkers.

There were significant correlations between the milking rates of dams and daughters and between the rates of sibs. From these results, and from the striking demonstration of the effect of the sire on the milking rates of his daughters, it is apparent that milking rate is inherited.

We are particularly indebted to Dr G. L. Bailey for considerable help and for advice on the methods of analysis used in this study. We would also like to acknowledge the help of Miss Ettie Henriques, Miss Eleanor Baxter and Mr P. A. Clough.

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637.127.1

507. VARIATIONS IN THE FAT CONTENT OF MILK THROUGHOUT THE MILKING PROCESS

By W. G. WHITTLESTONE

Ruakura Animal Research Station, Department of Agriculture, Hamilton, New Zealand

(With 3 Figures)

It is well known that the fat content of cow's milk increases as the cow is being milked. Crowther (1) showed that this effect is reduced as the period between what is now known to be let-down, and actual milking is increased. Johansson (2) has described the effect in detail and has measured the influence of a number of factors on this change in composition. He has shown that the rise in fat percentage is greatest in cows giving a large yield of milk, or milked after comparatively long intervals. Incomplete milking, or 'super milking' with the aid of Pitocin, were shown to have little effect on the average fat percentage measured over a period of several days. These experiments suggest that abnormally high or abnormally low intramammary pressures do not preferentially decrease fat secretion, any pressure effect apparently acting on both fat and serum in the same way. The writer has found (3) that the distribution of fat globule sizes does not usually change greatly throughout the milking of the cow.

In contrast with the cow, the sow shows no trend in the fat content of her milk throughout milking, nor is there any definite change in the fat globule distribution (4). Microscopic examination shows that the globules in sow's milk are somewhat smaller than those of cow's milk, and do not appear to cluster. Sow's colostrum is different in this respect, in that the globules cluster and, unlike sow's normal milk, the colostrum creams readily.

Because of the differences between sow's and cow's milk, the writer has undertaken an examination of current theories explaining the trends in composition of cow's milk, and has modified one of the theories in an attempt to explain all of the facts. The following section is a brief review of theories at present in circulation.

The creaming theory. This theory (5, 6) suggests that the richness of cow's strippings is due to the cream being drawn last. If creaming in the udder could occur, it would certainly explain the effects observed, just as the non-creaming properties of sow's milk would explain the lack of a trend in the fat content of the latter. Two basic facts, however, make the theory untenable:

(1) Most of the cow's milk is held in the alveoli prior to let-down. The diameter of the filled alveoli is of the order of 40–80 units (7, 8) so that any creaming which occurs, does so within a small volume. When the alveoli contract at let-down, the contents of the many alveoli will be mixed so that creaming effects will be largely eliminated, except perhaps for the small number of alveoli which empty via a ductule leaving the bottom of the lumen.

(2) By milking a goat on its back after massaging the udder, Gaines (9) showed that the fat trend is still maintained, while Crowther (1) showed that the tendency for the fat

percentage to increase is lost for a considerable portion of the milking if the quarter concerned is milked some time after the milking process is started in another quarter.

The filtering theory. According to this theory (6, 9, 10) the large globules are filtered out from the milk by the ductules, so that the first drawn milk contains only small globules. The strippings, according to this theory, contain a high percentage of large globules. Despite the somewhat smaller size of globule, it would be expected that this effect would show in the sow. However, the most serious objection to the theory is the fact that in serially drawn cow's milk samples, in which a marked change in fat percentage occurs, there is no corresponding trend in fat globule size distribution (see later).

The 'adsorption' theory. Swanson & Hinton (11) have suggested that the fat is more firmly held to the gland surfaces than are the other constituents. The observations on sow's milk, and the fact that the surface of the fat globules is hydrophilic and so no more likely to be adsorbed by a gland surface than, say, casein, make this theory improbable.

The pressure theory. The most generally accepted theory at the moment is Turner's pressure theory (12). According to this, as intra-alveolar pressure rises due to secretion, a point is reached at which the fat globules find their passage through the basal membrane into the lumen of the alveolus impeded. During let-down, when intra-alveolar pressure falls, this fat is released so giving rise to high fat strippings. Some objections to this theory are:

(1) An histological examination of a filled mammary gland (7, 8) suggests that it is unlikely that all of the fat contained in strippings could be held within the volume occupied by secreting tissue. It is not uncommon for 50% of the fat obtained at a normal milking to be obtained during the drawing of the last 20–25% of the milk.

(2) As the pressure within the alveolus increases the secreting cells are stretched and flattened, so presenting a larger area of basal membrane to the lumen. This should, if anything, increase the permeability of the membrane. Further, as the sustaining elements of the gland are behind the secreting cells, there can be no great increase in pressure across the basal membrane so that this could not be a factor in retaining the fat. The suggestion that the permeability of the basal membrane may be increased for some constituents by distension, is supported by the observation that pH and electrical conductivity of Pitocin 'super-strippings' tend to be higher than with normal strippings (13).

(3) It is in the interests of economy of hypotheses, to have a theory which will fit both cow and sow. It is also desirable to have a theory which would link the fact that milks which increase in fat content as milking proceeds, e.g. human (14), and goat (9), also cream, while sow's milk shows neither effect.

A modified filtration theory. The aim of the present paper is to suggest that the facts described above may be explained by assuming that the fat globules in the milk of the cow (and the human, goat, etc.) cluster within the alveoli. These clusters will be partly filtered out by the ducts, so that the last milk to leave the alveolus will be very rich in fat.

The facts to be fitted into the theory are:

(1) Cow's milk increases greatly in fat content as milking proceeds. Pitocin 'super-strippings' are very rich in fat.

(2) Sow's milk shows no such trend and 'super-strippings' are of similar fat content to normal milk.

(3) The goat, which shows an increase in fat content throughout milking, still does so when milked on its back after massage of the udder.

(4) There is probably not enough room in the secreting cells for the fat which may be removed as strippings and 'super-strippings' (15).

(5) Cow's milk creams and the globules cluster on standing at body temperature (see below).

(6) Sow's milk does not cream significantly under the same conditions.

(7) The change in fat content of cow's milk is greatest when the yield is high.

(8) Variations in intramammary pressure, prior to milking, do not affect the average fat content of cow's milk over a period.

(9) There is no significant change in the fat globule size distribution pattern of either cow or sow milk as milking proceeds.

It is suggested that there is a connexion between the fact that milk which creams also becomes richer in fat towards the end of milking. Rahn (16) has shown that the average-sized fat globule in cow's milk will rise due to gravity at 1–2 mm./hr. Creaming is a much more rapid process than this and may be explained by the clustering of the globules. Causes of clustering are reviewed by Dunkley & Sommer (17). The most likely theory assumes the existence of an agglutinin which causes the globules to stick together. This agglutinin is much more strongly adsorbed on the surface of solid globules than when they are liquid. Espe (18) concluded because of this, that the possibility of clumping at body temperature 'is altogether too remote to be considered'. However, there is no reason to assume a complete lack of adsorption of the agglutinin on liquid globules, though it is clear that the amount is greatly reduced compared with the concentration on solid globules.

It should be noted that the theory does not exclude the possibility that some slight filtering out of larger globules may occur. It is possible that cow's fore-milk may contain smaller globules than the rest of the milk due to slow filtering occurring between milkings, a situation which does not arise with the sow. So far this effect has not been established but is being investigated. The essential point of the theory under discussion lies in suggesting that the rapid rise in the fat content of the strippings is due to the filtering out of clusters within the alveoli. The theory depends on whether or not clustering or clumping does in fact occur in milk at 37° C.

EXPERIMENTAL

The creaming rate of warm cow's milk

In Exps. 1 and 2 the milk was drawn from the cow into a warm Thermos bottle, transferred quickly to creaming tubes and maintained at 37° C. in an incubator. The creaming tubes were 14 cm. long, 2 cm. in diameter, and fitted with a cock at the bottom so that the lower layer could be drawn off without disturbing the cream layer.

Exp. 1. Creaming in fore milk of 1·6% fat. The depth of the cream layer and fat content of the lower layer for three different creaming times are given in Table 1.

Exp. 2. Creaming in strippings of 8·9% fat. The results of the experiment are set out in Table 2.

Table 1. *Creaming in fore-milk at* 37° C.

Time (hr.)	2	4	9
Depth of cream layer (cm.)	0·5	0·5	0·6
% fat in lower layer	1·0	0·9	0·7
% fat in whole milk	1·6	—	—

Table 2. *Creaming in strippings at* 37° C.

Time (hr.)	1	2·5	5·5
% fat in lower layer	6·7	5·5	4·7
% fat in whole milk	8·9	—	—

In Exp. 1, 38% of the fat moved a mean distance of 7 cm. at 45 mm./hr., 44% at 18 mm./hr., and 56% at 7·8 mm./hr. The creaming rate of 25% of the fat in Exp. 2 was 70 mm./hr. This rate corresponds to a globule size of 25 μ based on Stokes' law.

$$V = \frac{2ga^2(d_1 - d_2)}{9\eta},$$

V = particle velocity in cm./sec.

a = radius of particle in cm.

d_1, d_2 = densities of milk and butterfat = 1·04 and 0·9 respectively.

η = viscosity of skim milk = 0·0172 poises.

g = acceleration due to gravity.

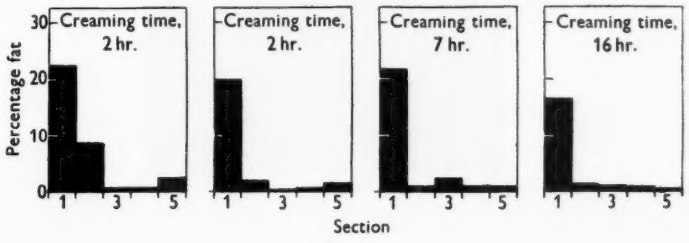

Fig. 1

In Exps. 3 and 4 a different type of creaming tube was used. It consisted of five sections 65 mm. long and 19 mm. diameter. The bottom section was, of course, closed at the lower end and the five sections were fitted together with rubber sleeves. The tube was almost filled with milk and creaming carried out at 37° C. At the end of the creaming period the tube was plunged into alcohol at −12° C. in a freezer. The frozen contents were then divided into sections, thawed and the fat content determined.

Exp. 3. Cow's milk maintained at 37° C. was placed in the segmented creaming tube for 16, 7 and 2 hr. and the fat distribution determined. The results are set out in Fig. 1. For the two 2 hr. tests 84 and 80% of the fat respectively moved with a mean velocity of approximately 80 mm./hr. This corresponds to a particle with a diameter of 27 μ and so represents a substantial degree of clustering.

Exp. 4. Sow's milk was subjected to creaming tests similar to those described in Exp. 3. Fig. 2 shows the results. It is quite evident that there is little creaming in sow's milk and therefore little clustering of the globules.

In order to ensure that the small globule size of sow's milk was not the factor controlling creaming rate, the following experiments were carried out. The creaming cells used in this case were $2 \times 3 \times \frac{1}{16}$ in. perspex cells similar to those described by Dunkley & Sommer (17). These cells made it possible to detect creaming qualitatively. In order to see the fat layer more readily, the milk was dyed with malachite green which has been shown to have no effect on creaming.

Exp. 5. A sample of sow colostrum was taken 24 hr. after parturition. At this stage the viscosity is almost normal, but the globulin fraction is still high. As a control a sample of normal sow's milk was placed in a similar cell and both left for 3 hr. in a 37° C. incubator. A clearly defined 7 mm. cream layer was evident in the colostrum sample while no layer could be detected in the normal milk.

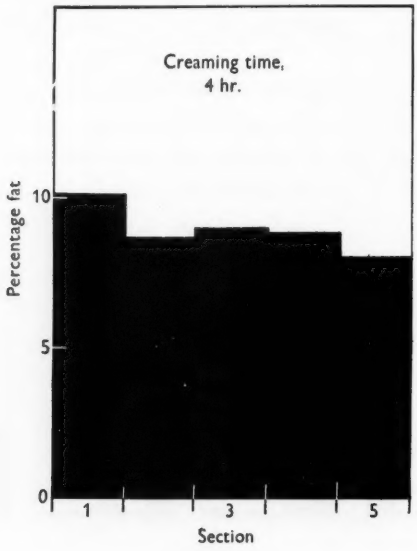

Fig. 2

Exp. 6. A sample of sow's milk was centrifuged and the cream layer removed. Fresh cow's milk was centrifuged warm and the skim-milk carefully separated. The sow's milk fat was then dispersed in the cow skim-milk to give a uniform suspension. The composite milk was placed in a creaming cell. After 1 hr. a distinct cream layer had formed on the milk, while a sample of the original sow's milk similarly treated did not show a detectable cream layer until about 16 hr. had elapsed. It would appear that sow's milk will cream readily if an agglutinin is supplied.

A second important question is whether or not there is any evidence for the filtering out of large globules, so that the richness of strippings may be attributed to an increase in fat globule size. A study has been made of the fat globule size distribution of cow's milk. Fig. 3 shows a typical set of size distribution patterns. It is evident that no marked change in fat globule size distribution occurs in cow's milk as it is being withdrawn. This is perhaps not surprising as no clear trends are shown in sow's milk, despite its containing a wide range of fat globule sizes (4).

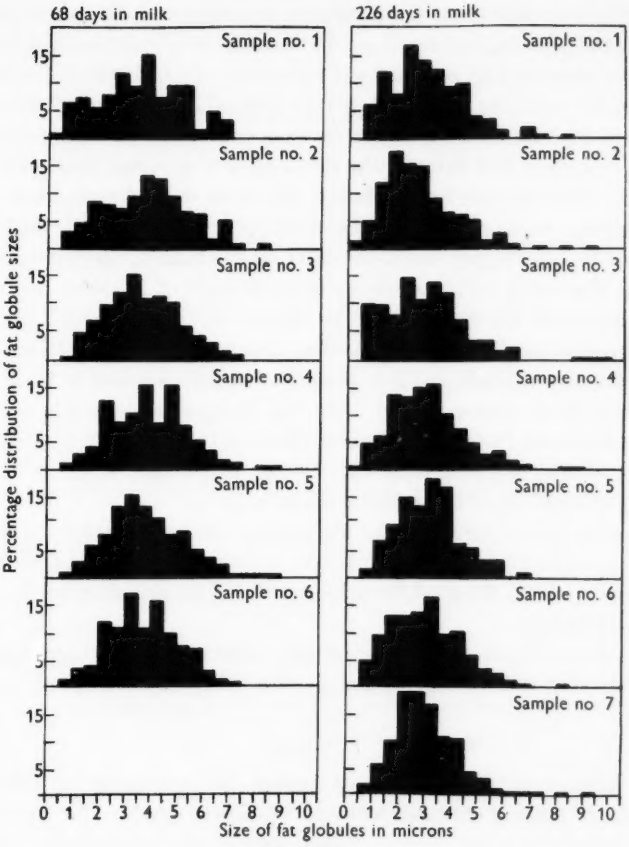

Fig. 3

DISCUSSION

Johansson (2) has stated: 'The conclusion may be drawn that the rate of fat secretion, in proportion to the secretion of milk serum, is very little changed by variations in the degree of udder evacuation. Calculated as an average for several days in succession, the fat percentage of the milk is practically independent of the completeness of milking.' This is not in agreement with the earlier view of Hammond (10) that, 'the increase in the milk pressure in the udder inhibits the production of fat to an even greater extent than it inhibits the yield of the milk'. That high intramammary pressures have a specific effect on the release of fat from the milk-secreting cells, is the basis of Turner's theory (12) explaining the high fat content of strippings. This view has been used to explain Hammond's concept, because the accumulation of fat rather than serum within the cells may preferentially inhibit the secretion of the former. The 'law of mass action' has been suggested as explaining this effect.

Johansson's results make it clear that pressure effects on secretion do not differentially affect fat secretion. Hence it is desirable to seek an explanation for the increasing fat content of cow's milk, in terms not involving this effect. It is thought that by assuming

that fat globules may cluster within the alveoli, an assumption for which some evidence has been presented, the resultant tendency for the clusters to be filtered out by the ductules will explain the increasing fat content, while avoiding effects due to the specific accumulation of fat in the secreting cells of cow's milk throughout milking. Further, when it is realized that the high-fat milk left behind by such a filtering process has a high viscosity, and hence a low rate of flow through the ductules, it is apparent that if the duration of let-down is not great enough to enable the cow to be milked completely at a normal milking, the theory explains the presence of strippings of abnormally high fat content. There is reason to believe that when the dairy cow is milked, the duration of let-down varies. This is associated with the release of an amount of let-down hormone which is substantially less than the doses needed to extract 'super-strippings'. In the sow it is known that the amount of hormone released at a normal suckling is between 0·5 and 1 i.u. of posterior pituitary extract (19). It is probable that the amount of let-down hormone released at a milking varies greatly with the changes in stimulus associated with milking (20). This would have a considerable effect on the efficiency of udder evacuation, and while not causing any long-term effect on fat percentage, would be responsible for considerable variations from one milking to the next.

The explanation offered associates the ability to cream with the trend in fat throughout milking by suggesting that these factors have a common cause—the clustering of the globules—while it avoids the need for a special theory to explain why the sow does not show fat-rich strippings.

The experiments described provide further evidence for the agglutinin theory of creaming by showing that the agglutinin of cow's milk will cause the clustering of sow's milk globules.

SUMMARY

The various theories commonly used to explain the increasing fat content of cow's milk with stage of milking are examined and found inadequate. It is suggested that the trends in fat content as a cow is being milked may be explained by the clustering of the fat globules and the consequent partial filtering out of the clusters as the milk flows from the gland. Evidence for clustering of fat in cow's milk at body temperature is presented and the application of the theory to the sow discussed.

The writer is indebted to Mrs D. R. Perrin for the fat analyses involved in this work, to Messrs D. M. Smith and E. D. O'Reilly for sow's milk.

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508. NOTE ON THE CORROSION OF ALUMINIUM DAIRY EQUIPMENT BY WATER

By G. H. BOTHAM and W. R. BRYSON

A.P.V. Laboratories, London

(With 1 Plate)

Some few months ago the National Agricultural Advisory Service, South West Sub-Centre, encountered several farms in their area where failure to reach the required standard for designated milk appeared to be connected in some way with pronounced pitting corrosion of aluminium alloy milking buckets, and, on the suggestion of Dr A. R. T. Mattick of the National Institute for Research in Dairying, Reading, the authors of this note were given the opportunity of examining a corroded pail and of tracing the source of the corrosion, if possible.

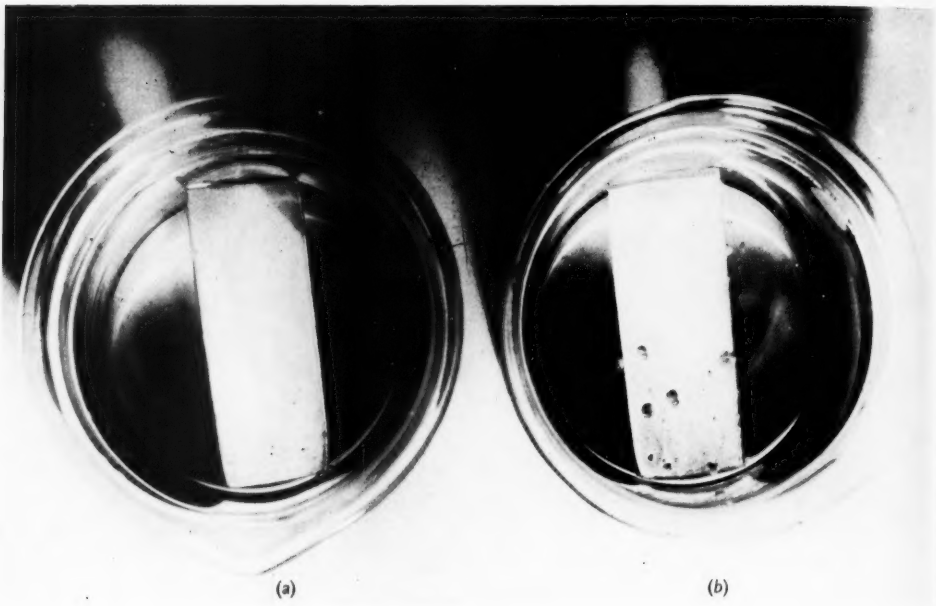
The attack consisted essentially of pitting, the pits being surrounded by white corrosion product. This corrosion product contained milk protein, and it appeared quite likely that a simple hypochlorite wash would fail to penetrate such material and in this way the efficiency of sterilization could be impaired.

However, the reported details of the methods of use of detergents and hypochlorites did not appear to the authors to be such as to lead to rapid corrosion, and attention was concentrated on the water supplies used for rinsing and especially the quality of the water which would remain in contact with the buckets overnight.

In the most striking case examined, the farm concerned drew its water from a well. Extensions made in recent years were served by copper water pipes, while the old intallation was of lead. Samples of water passing through the two sets of pipes were of very similar character, with the exception of their dissolved copper content. The water passed through the copper pipe was found to contain 7·8 p.p.m. of copper, while that which had been in contact with the lead contained less than 0·01 p.p.m. copper. A simple full immersion test on aluminium test pieces demonstrated that typical pitting attack began in 6 hr. when the copper-containing water was used, yet in 3 days the water that had passed through lead caused hardly any corrosion. Given the same rate of corrosion in practice, a milking pail rinsed with the first water and left standing wet overnight would be covered by small nodules of white corrosion product in the morning.

Other cases where corrosion of this nature has occurred disclosed dissolved copper contents varying between 4 and 1 p.p.m., and there can be no doubt that the corrosion trouble was largely due to this cause. The effect of coupling of dissimilar metals such as copper and aluminium is very widely known, but the fact that the precipitation of dissolved copper from aqueous solution by an aluminium surface may accelerate corrosion is often overlooked.

Some attempts have been made to provide a method of removing dissolved copper from water by simple means. The first trial consisted in passing the water over a large surface of aluminium in the form of a column of rivets. The results, collected in Table 1, were plainly disappointing.



24 hr. full-immersion test at room temperature. *Aluminium:* ordinary purity.

(*a*) Water passed through Zeocarb 215 at 2400 ml./min. and containg 0·03 p.p.m. dissolved copper (97% copper removal).
(*b*) Original water containing 1·62 p.p.m. dissolved copper.

Table 1. *Aluminium column of aluminium rivets* ($\frac{1}{2} \times \frac{3}{16}$ in.) *packed in a vertical glass tube to form a column* 16 in. *long* × 2 *in. diam. Tap water containing* 1 *p.p.m. added copper was allowed to pass down the column at varying rates and the effluent was analysed*

Throughput (ml./min.)	Copper in feed (p.p.m.)	Copper in effluent (p.p.m.)	% removal of copper
7·9	0·96	0·40	58
70	0·96	0·64	33
540	0·96	0·77	20
1000	0·96	0·84	12

The next approach was much more successful. A column filled with a synthetic resin (Zeocarb 215—supplied by the Permutit Co. Ltd.) appeared to be capable of removing dissolved copper at a practicable rate and to a surprisingly high degree, as shown in Table 2.

Table 2. *Zeocarb* 215, *prepared as directed by the Permutit Co. Ltd., was packed into a vertical glass tube to form a column* 15 *in. long* × 2 *in. diam. and tap water containing* 1 *p.p.m. added copper was passed down the column as before*

Throughput (ml./min.)	Copper in feed (p.p.m.)	Copper in effluent (p.p.m.)	% removal of copper
450	1·02	<0·01	>99
1000	1·02	<0·01	>99
2400	1·02	0·03	~97

Pl. 1 shows how the passage, at an appreciable rate, of water containing originally 1 p.p.m. of dissolved copper affects its corrosiveness to aluminium in a 24 hr. test, and it is probable that this method would afford an appreciable degree of protection.

No attempt has been made by the present authors to extend the method to actual aluminium equipment in service, but it is thought that the technique of using and regenerating a bed of resin to remove dissolved copper from supply waters which are to come in contact with aluminium should be neither difficult nor unduly expensive, for it is very similar to that employed with zeolite water softeners, which are in very wide use.

While it is recognized that the above observation might not be of any practical use to the farmer, it is thought that the owner of large aluminium vessels may derive benefit from the application of the above idea.

The authors wish to thank the Directors of the A.P.V. Co. Ltd. for permission to publish this note.

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509. BACTERIOLOGICAL STUDIES ON PASTEURIZED MILK

By HAZEL ALEXANDER and CONSTANCE HIGGINBOTTOM
The Hannah Dairy Research Institute, Kirkhill, Ayr

(With 4 Figures)

In 1941, Rowlands & Provan(1) described the changes which took place in 'in bottle' pasteurized milk during storage at 15·5° C. They found that acid-producing bacteria predominated immediately after pasteurization and that these were superseded after 48 hr. at 15·5° C. by alkali-producing and peptonizing bacteria. After longer storage acid-producing bacteria which differed from those originally dominant were the main species and were finally responsible for the spoilage of the pasteurized milk.

An investigation of the changes taking place during the storage of milk pasteurized by the high-temperature short-time (H.T.S.T.) process has now been started. Before beginning this investigation it seemed desirable to determine the types of bacteria normally present in H.T.S.T. pasteurized milk as it left the pasteurizer and before it became liable to post-pasteurization contamination during the bottling process, and to compare the microflora with that in the corresponding raw milk and in this milk after pasteurization by the low-temperature (L.T.) 'Holder' process. Since it was impracticable to obtain 'Holder' pasteurized milk prepared on a commercial scale from the same raw milk as that subjected to H.T.S.T. pasteurization this milk was obtained by pasteurization in the laboratory for 30 min. at 62·5–63° C.

The present survey was made between November 1948 and January 1951. Shortly before the completion of the work two papers were published by Egdell & Bird(2, 3) and one by Ashton(4) giving results of similar investigations. The first part of this paper will therefore give a brief report of the results of the present survey, with particular reference to the work already published on this subject. In the second part of the paper details will be given of the preliminary observations made on the microflora associated with the deterioration of H.T.S.T. and the corresponding L.T. pasteurized milks when stored at 18·5 and 6° C.

METHODS OF SAMPLING

Samples of pasteurized milk were obtained directly from the cooling section of four commercially operated H.T.S.T. pasteurizing plants. These samples were subject to any post-pasteurization contamination arising in the regeneration and cooling sections of the heat exchanger, but further contamination during the bottling process was avoided. No attempt was made to interfere with the normal operation of the plants, but samples were taken only when the plant appeared to be operating correctly.

The raw milk samples were obtained either from the holding tank, in which case the flow of raw milk to the tank was stopped until after the pasteurized milk had been sampled, or from the float-controlled balance tank, when care was taken to avoid sampling when any rejected milk was being returned from the pasteurizer. The sampling of the pasteurized milk was timed so that as far as possible it corresponded to the raw milk already sampled.

The milks were examined within 1 hr. of sampling. For the laboratory pasteurization 300 ml. of the raw milk in a 500 ml. flask were heated at 62·5–63° C. for 30 min., the flask being immersed in a water-bath to within 1 in. of the neck and the time of heating determined using a similar control flask fitted with a thermometer. The time required to reach 62·5° C. was 12–15 min. The L.T. pasteurized milk was immediately cooled in running tap water.

METHODS OF EXAMINATION

Phosphatase test. All pasteurized milk samples were subjected to the Kay and Graham test (5).

Colony counts. Duplicate plates on 'Yeastrel' milk agar (6) were incubated at 37° C. (3 days), 30° C. (5 days) and for a limited number of samples at 56° C. (2 days).

Coliform test. The presumptive test (6) at 37° C. was made on 1, 0·1 and 0·01 ml. of both raw and pasteurized milks and the positive tests confirmed by plating on McConkey agar.

Titratable acidity. 10 ml. milk containing 1 ml. phenolphthalein (0·05% in 50% ethanol) was titrated with N/9-NaOH to an end-point matching the colour obtained by the addition to 10 ml. of milk of 1 ml. of a freshly diluted solution (0·0005%) of rosaniline hydrochloride. The titratable acidity was expressed as ml. N/9-NaOH required to neutralize 10 ml. milk.

Keeping quality tests

Dye-reduction test. All milks were subjected to a methylene blue keeping quality test at atmospheric shade temperature. The pasteurized milks were also examined by the methylene blue test at 37° C. after storage overnight at atmospheric shade temperature (7).

Alcohol test. 1 ml. of 68% ethanol (neutralized) containing 0·02% methyl orange was added to 1 ml. milk. The colour change was compared with a control tube adjusted to a titratable acidity of 2·8 and the results graded according to Anderson & Wilson (8), i.e. grade I, no precipitate, grade II, precipitation with acidity under 2·8 (sweet clot) and grade III precipitate with acidity 2·8 or over (acid clot).

Clot-on-boiling. A positive test was recorded when any precipitate was formed in 3 ml. milk in a test-tube immersed for 5 min. in boiling water.

Microflora. The predominating micro-organisms in the milk were determined by picking colonies from two or more opposite sectors of a countable plate. Usually fifteen to twenty colonies were transferred from each plate and the bacteria characterized and, where possible, identified. Streptococci were identified using the classification of Sherman (9), the aerobic spore-forming bacilli according to that of Gibson & Topping (10) and coliform bacteria in accordance with Wilson and his co-workers (11).

RESULTS

A. *A comparative study of the numbers and types of bacteria surviving in commercial high-temperature short-time and laboratory low-temperature pasteurized milks and in the corresponding raw milk*

Colony counts

Growth of thermophilic bacteria at 56° C. was obtained in only three of the seventeen samples of H.T.S.T. pasteurized milk examined. These three samples were all obtained from one plant, and four other samples from the same plant contained no thermophiles.

No other samples of H.T.S.T. pasteurized milk, nor the seventeen corresponding samples of L.T. pasteurized milk gave any growth on plates incubated at 56° C. The incidence of thermophilic bacteria was not further investigated.

The geometric mean count at 37° C. for sixty-one samples of H.T.S.T. pasteurized milk and the corresponding raw milks was 7,410 and 223,870 per ml. respectively, and the corresponding figures after incubation at 30° C. were 13,800 and 321,130 per ml. The reduction in count due to H.T.S.T. pasteurization was 96·7 and 95·8% for the microflora developing at 37° and at 30° C. respectively.

Twenty-five of these raw milks were subjected to L.T. pasteurization. The comparative figures for the geometric mean count at 37 and 30° C. are given in Table 1 for these

Table 1. *The effect of laboratory* L.T. *pasteurization* (*L*) *compared with that of commercial* H.T.S.T. *pasteurization* (*H*) *on the numbers of bacteria present in raw milk* (*R*) *and developing on Yeastrel milk agar plates incubated at* 37 *and* 30° C.

Plant	No. of samples	Geom. mean count			Destruction of bacteria during pasteurization (%)	
		R	L	H	L	H
			(A) 37° C.			
A	6	60,260	440	1,820	99·3	97·0
B	6	117,490	1,950	3,550	98·3	96·9
C	6	53,700	1,510	2,750	97·2	94·9
D	7	81,280	3,160	7,940	96·1	90·2
Total	25	74,130	1,740	3,200	97·7	95·9
Ashton(4)	36	649,400	10,090	19,470	96·5	95·1
Egdell(2)	21	257,000	1,670	2,330*	99·4	99·1
			(B) 30° C.			
A	6	112,200	1,740	3,090	98·5	97·3
B	6	199,530	10,000	14,130	95·0	92·9
C	6	89,130	4,800	7,410	94·6	91·7
D	7	117,490	16,980	20,890	85·6	82·2
Total	25	123,030	7,080	8,510	94·3	93·1
Ashton(4)	36	2,985,000	33,960	58,430	97·3	96·7
Egdell(2)	21	—	11,600	9,200*	—	—

* Includes results from five H.T.S.T. and six L.T. pasteurization plants.

twenty-five samples of raw, H.T.S.T. and L.T. pasteurized milks, and the figures obtained by Egdell & Bird(2) and by Ashton(4) have been included for comparison. The results confirmed those of Ashton and of Egdell & Bird, and showed that the percentage destruction of the bacteria was slightly greater in the L.T. than in the H.T.S.T. pasteurized milks, and that it was of the same order as that obtained by Ashton, although he was using raw milk of much higher bacterial content.

Coliform test

All but three of sixty-one raw milks gave a positive presumptive coliform test, and thirty-nine (62%) were positive in 0·01 ml. Twelve of the corresponding H.T.S.T. pasteurized milks gave a positive presumptive coliform test, ten of these being from one plant (A). No coliforms were present in 1 ml. of the twenty-five L.T. pasteurized milks. Ashton(4) and Egdell & Bird(2) obtained a negative coliform test with all pasteurized milks. Although samples were taken direct from the pasteurizer it seems probable that at plant A some post-pasteurization contamination arose in the cooling section of the plant since in only three of the milks was the same type of coliform bacterium isolated from both the raw and the pasteurized milk.

Keeping quality

Methylene blue test. All samples of pasteurized milk passed the standard test (7) in which the milk is held overnight at atmospheric shade temperature and then incubated at 37° C. for 30 min. Observations were continued up to 8 hr. at 37° C. Of sixty-one H.T.S.T. pasteurized milks fourteen reduced methylene blue after 2–8 hr. at 37° C., but all twenty-five samples of L.T. pasteurized milk had dye reduction times greater than 8 hr. There was some suggestion of a relationship between the reduction time of 2–8 hr. and a high colony count and a positive coliform test but this was not clear-cut.

When duplicate tests were carried out entirely at atmospheric shade temperature, no pasteurized milk reduced the dye in 24 hr., but ten samples had done so after 2 days, and of these, eight had reduced methylene blue within 8 hr. at 37° C. There appeared, therefore, to be little difference between the two methods of carrying out this keeping quality test.

Clot-on-boiling and alcohol tests. The mean time at which H.T.S.T. and L.T. pasteurized milks (twenty-four samples) held at 18·5° C. became unpalatable or clotted on boiling was 3·0 and 3·2 days respectively. In fifteen samples (62·5%) the keeping quality of the H.T.S.T. and L.T. pasteurized milks did not differ by more than half a day (tests were made twice a day). Eleven samples had a longer and six samples a shorter keeping quality after L.T. than after H.T.S.T. pasteurization.

Similar results were obtained when the alcohol test was used to assess the keeping quality. The keeping quality determined by alcohol and by taste and clot-on-boiling was identical for twenty of the twenty-four samples of both H.T.S.T. and L.T. pasteurized milks. Ashton (4) also found a high degree of correlation between these tests. He found the keeping quality of the H.T.S.T. pasteurized milks to be slightly longer than that of the L.T. pasteurized milks, although the initial plate count was higher in the milks pasteurized at the higher temperature.

The microflora of the raw and freshly pasteurized milks

The reactions in litmus milk of the predominating flora in twenty-two samples of raw milk and the corresponding samples of H.T.S.T. and L.T. pasteurized milks are shown in Table 2. In all samples acid-producing bacteria predominated, and in the pasteurized

Table 2. *The reaction in litmus milk of the bacteria isolated from Yeastrel milk agar plates incubated at* 37 *and* 30° C. *from twenty-two samples of raw milk and the corresponding* L.T. *and* H.T.S.T. *pasteurized milk*

Temp. of incubation (° C.)	Type of milk	Reaction in litmus milk. Percentage of colonies giving				
		Acid	Acid-clot	Alkaline	Peptonization	Inert
37	R*	20	61	7	7	5
	L	43	47	2	6	2
	H	36	59	1	3	1
30	R	17	51	17	4	11
	L	22·5	73·5	1·5	0·5	2
	H	28	66·5	2	0·5	3

* R, raw; L, L.T. pasteurized milk; H, H.T.S.T. pasteurized milk.

milk they constituted over 90% of the bacteria. The proportion of these bacteria, which in pure culture did not produce sufficient acid to clot the milk, was higher in the pasteurized milks and greater for those developing on plates incubated at 37° C. than at

30° C. The proportion of non-acid producing bacteria was lower in the pasteurized than in the raw milks. There was no appreciable difference in the reactions in litmus milk between H.T.S.T. and L.T. pasteurized milks. These results are similar to those obtained by Ashton(4), but show a higher proportion of strains producing acid without clotting the milk at 37° C. though not at 30° C., and in the raw milk a higher proportion of alkali-producing bacteria and a lower proportion of peptonizing strains in the flora developing at 30° C.

The percentage distribution of the different groups of bacteria developing at 37 and at 30° C. is given in Table 3. Streptococci predominated in the raw milks but were much

Table 3. *The percentage distribution of different groups of bacteria in twenty-two samples of raw and* L.T. *and* H.T.S.T. *pasteurized milks*

Temp. of incubation (° C.)	Type of milk	Strepto-cocci	Micro-cocci	Micro-bacteria	Aerobic spore-forming bacilli	Non-sporing bacilli*		Yeasts and other species
						Alkali producers	Others	
37	R†	62·25	19	3	0·75	8	7	0
	L	22·25	45·5	22	7·75	2	0·25	0·25
	H	25·5	39	27	4·5	2	1·5	0·5
30	R	45·5	15	9	2·5	18	10	0
	L	5	21	70	1·75	0·5	1	0·25
	H	9·5	16	67·5	2·75	2	2·25	0

* Excluding microbacteria.
† R, raw; L, L.T. pasteurized; H, H.T.S.T. pasteurized.

less numerous in the pasteurized milks. After pasteurization there was an increase in the proportion of microbacteria on plates incubated at both temperatures but especially so at 30° C. The proportion of micrococci was markedly increased on the plates at 37° C. but not at 30° C., and there was a considerable decrease in the non-sporing rods on plates at both incubation temperatures. Apart from the slightly lower proportion of streptococci in the L.T. pasteurized milks there was little difference in the proportions of the various groups of bacteria in the L.T. and the H.T.S.T. pasteurized milks.

Table 4. *The estimated mean numbers of various groups of bacteria in raw and pasteurized milk calculated from the geometric mean count and the percentage distribution of the different groups*

Temp. of incubation (° C.)	Type of milk	Geom. mean count/ml.	Strepto-cocci	Micro-cocci	Micro-bacteria	Spore-forming rods	Non-spore-forming rods	Yeasts and other species
37	R*	72,440	45,095	13,763	2,173	543	10,866	0
	L	1,200	267	546	264	93	27	13
	H	2,950	752	1,451	796	133	103	15
30	R	125,900	57,295	18,885	11,331	3,147	35,252	0
	L	6,310	316	1,325	4,417	110	126	16
	H	8,500	807	1,360	5,738	234	361	0

* R, raw; L, L.T. pasteurized; H, H.T.S.T. pasteurized.

Although there was an increase in the proportion of micrococci and microbacteria in the pasteurized milks, there was of course an actual decrease in the numbers of these bacteria since the total plate count was much lower in the pasteurized than in the raw milks. The estimated mean numbers of the various groups of bacteria are given in Table 4. In the pasteurized milks the percentage of surviving bacteria in each group, compared with those estimated to be present in the raw milks, was much greater in the micro-

bacteria and the spore-forming rods than in the other groups, and greater in the H.T.S.T. than in the L.T. pasteurized milk for all groups except the micrococci (Table 5).

Table 5. *The surviving bacteria of each group in the pasteurized milk expressed as a percentage of the estimated numbers in the raw milk*

Temp. of incubation (° C.)	Type of milk	Surviving bacteria (%)					
		Total	Streptococci	Micrococci	Microbacteria	Spore-forming rods	Non-spore-forming rods
37	L*	1·7	0·6	4·0	12·2	17·1	0·3
	H	4·1	1·7	1·1	36·6	24·5	1·0
30	L	5·0	0·6	7·0	3·9	3·5	0·4
	H	6·0	1·4	7·2	5·1	7·4	1·0

* L, L.T. pasteurized; H, H.T.S.T. pasteurized.

The number of samples from which particular groups of bacteria were isolated (irrespective of the numbers in each group) are given in Table 6. Streptococci of the *lactis-cremoris* group were present in all but one of the raw milk samples, and most raw milks also contained *Streptococcus faecalis*. No streptococci were isolated from about half the pasteurized milks, but from the remainder *Str. thermophilis* and from a few samples *Str. faecalis* were obtained. *Str. bovis* was not isolated from the pasteurized milks.

Table 6. *The incidence of specific groups of bacteria in twenty-two samples of raw and pasteurized milks*

Groups of bacteria	No. of samples from which species were isolated		
	Raw milk	Pasteurized milk	
		L.T.	H.T.S.T.
Streptococci			
lactis-cremoris group	21	—	—
faecalis	16	3	1
thermophilus	—	8	12
bovis	1	—	—
kefir	—	—	1
unidentified	2	1	1
Micrococci			
producing acid	13	16	19
acid, slow clot	2	9	10
acid and clot	10	3	4
acid, clot and peptonization	5	1	2
alkali	1	2	1
peptonization (no acid)	2	1	—
inert	12	4	4
Microbacteria	15	21	21
Non-sporing rods			
producing acid	5	2	6
alkali	21	4	6
peptonization	10	—	1
inert	7	5	4
coli-aerogenes group	3	—	—
Spore-forming rods			
B. subtilis	1	2	2
B. pumilus	3	4	3
B. licheniformis	—	—	1
B. circulans	—	1	1
unidentified	4	4	

Most milks, whether raw or pasteurized, contained acid-producing micrococci, but the incidence of micrococci producing acid in sufficient amount of clot milk was lower and that of the weak acid-producers higher in the pasteurized than in the raw milks.

Microbacteria were present in all but one of the pasteurized milks, and were also detected in small numbers in fifteen of the raw milks. Alkali-producing rods were present in all but one of the raw milks, but were isolated from only a few samples of pasteurized milk. These were, with the exception of a few strains of a chromogenic bacterium, all one species of *Alcaligenes*, probably *A. metalcaligenes*, i.e. non-motile coccobacilli which did not liquefy gelatin or produce acid from sugars, and might or might not reduce nitrate to nitrite. Although only small numbers of this species were isolated from freshly pasteurized milk they frequently (see part B of this paper) became the predominant species in pasteurized milks after storage at either 18·5 or 6° C.

The results obtained in this study of the microflora of raw and the corresponding L.T. (laboratory) and H.T.S.T. (commercial) pasteurized milks were substantially the same as those obtained for laboratory (L.T.) pasteurized milks by Gibson & Abd-el-Malek (12), by Egdell & Bird (3) for commercially processed H.T.S.T. and L.T. pasteurized milks, and by Ashton (4) for H.T.S.T. pasteurized samples taken direct from the holding unit and for the corresponding L.T. (laboratory) pasteurized milks. The predominating species of bacteria were the same in all these investigations, but differed somewhat in the relative proportion in each group, especially in the streptococci. This difference in the proportion of streptococci in the pasteurized milk appears to bear some relation to the quality of the raw milk. Egdell & Bird, starting with raw milk with a mean count (37° C.) of 257,000 per ml., found streptococci to constitute 49·9% of the microflora of the pasteurized milk, whereas in the present survey with raw milk of a mean count of 74,130 per ml. the proportion of streptococci after pasteurization was only 22–25·5%. Ashton, using raw milk of higher mean count (649,400 per ml.), reported that streptococci were present in considerable numbers in all samples, whereas streptococci were not detected in about half of the pasteurized milks described in the present paper. This confirms the earlier work of Gibson & Abd-el-Malek (12) who found that the streptococci present in six low-count certified milks were not detected after laboratory pasteurization at 63° C. for 30 min. but did constitute a high proportion of the surviving bacteria in five out of six samples of raw milk of high bacterial count.

In a further series of pasteurized milks Gibson & Abd-el-Malek (13) found the predominating species of *Streptococcus* to be *Str. thermophilis* associated with smaller numbers of *Str. bovis*; Egdell & Bird (3) found *Str. thermophilis* with *Str. bovis* only in samples of bottled pasteurized milk. Ashton (4) also isolated a few strains of *Str. bovis* but failed to isolate *Str. thermophilis* and he found *Str. faecalis* to be the predominating species. In the present survey *Str. thermophilis* was the *Streptococcus* most frequently isolated with small numbers of one strain of the *Str. faecalis* group but *Str. bovis* was not encountered.

B. *Preliminary studies of some of the changes taking place in pasteurized milk during storage at* 18·5 *and* 6° C.

Before attempting to investigate in detail and on a quantitative basis the changes in bacterial and chemical composition of pasteurized milks during storage, some observations were made which would give a general indication of some of the changes to be

expected. Twenty-four samples of H.T.S.T. pasteurized milks taken direct from the cooling section of the pasteurizer and of the corresponding raw milks after L.T. (laboratory) pasteurization were examined. One portion of each sample was held in a water-bath at 18·5° C. and the other in a refrigerator at approximately 6° C. Observations of the titratable acidity, palatability, stability to 68% alcohol and to boiling water were made twice daily (10 a.m. and 4 p.m.) on samples held at 18·5° C. and once each week on samples held at 6° C. The predominating microflora in seven of these samples of H.T.S.T. and the corresponding L.T. pasteurized milk was determined at the same intervals. For this purpose 'Yeastrel' milk agar plates were spread with 0·1 ml. of the milk (or a suitable dilution in quarter-strength Ringer's solution), incubated at 22° C. for 5 days or where required, at 6° C. for a maximum of 3 weeks, and the resulting colonies examined. For milks held at 6° C. the microflora developing on plates incubated at 6° C. was similar to that obtained on duplicate plates incubated at 22° C. and no micro-organisms were isolated at 6° C. which did not grow at 22° C.

Titratable acidity and keeping quality

Storage at 18·5° C.

The development of titratable acidity in samples of H.T.S.T. and L.T. pasteurized milks held at 18·5° C. is shown in Figs. 1 and 2. (Samples in which the predominating micro-

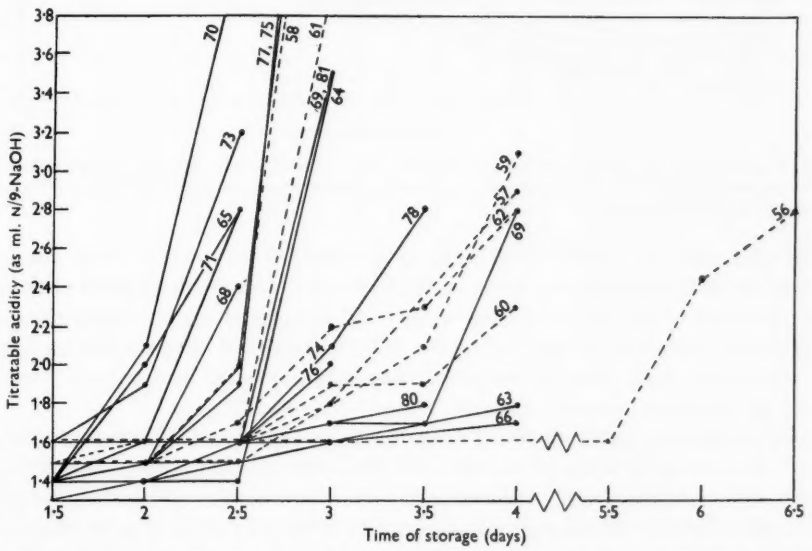

Fig. 1. The development of titratable acidity in twenty-four samples of H.T.S.T. pasteurized milk during storage at 18·5° C. Numbers: code no. of sample. Broken lines: samples in which the predominating microflora was determined.

flora has been determined are shown as broken lines for ease of recognition.) The difference in both the rate and the amount of acid production between the H.T.S.T. and L.T. pasteurized milks is shown clearly in these figures. Approximately half the samples of H.T.S.T. pasteurized milks showed a sudden rapid increase in titratable acidity after 2 or 2½ days at 18·5° C. The remaining samples showed a much more gradual increase in

acidity. In a few samples the acidity had not risen above 1·8 after 4 days, while in the others the rate of acid production tended to increase with length of incubation time. With the corresponding L.T. pasteurized milks the rise in acid production was slower, and with one exception (no. 75) no sample had a titratable acidity above 2·0 after 3 days at 18·5° C. Some samples did show a sudden increase in titratable acidity but not usually until 3–3½ days and such samples were fewer than in the H.T.S.T. pasteurized milks.

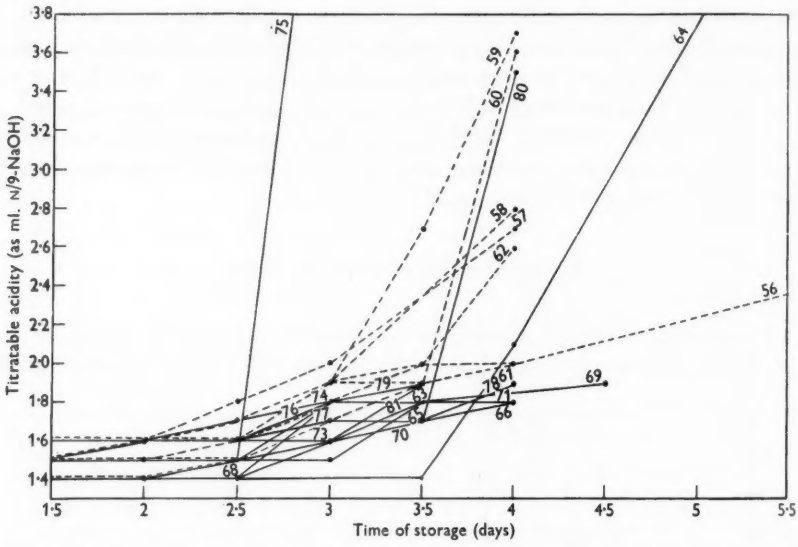

Fig. 2. The development of titratable acidity in twenty-four samples of L.T. (laboratory) pasteurized milk during storage at 18·5° C. Numbers: code no. of sample. Broken lines: samples in which the predominating microflora was determined.

The time taken to reach certain levels of titratable acidity and to show instability towards alcohol or on boiling is given in Tables 7 and 8. A brief description of the type of deterioration is also given in these tables. All samples of H.T.S.T. pasteurized milk developed an acidity of 1·8 and only seven failed to reach 2·8, whereas four samples of L.T. pasteurized milk failed to reach 1·8, and nineteen failed to develop a titratable acidity of 2·8 before becoming unstable to alcohol or to boiling.

The mean keeping quality assessed as the time at which the samples became unstable to 68% alcohol (grade II) or gave a clot on boiling was 2·9 and 3·0 days respectively for the H.T.S.T. pasteurized milks and 3·1 days (both tests) for the L.T. pasteurized milks. This small difference between the keeping quality of the two types of pasteurized milks emphasized the frequency with which the useful life of the milks was limited by the changes leading to instability rather than to acid production. Many of the H.T.S.T. pasteurized milks were at acidities between 1·8 and 2·8 when 'sweet' clotting occurred, and this was frequently followed by further acid production on continued incubation whereas with the L.T. pasteurized milks instability to both alcohol and boiling occurred at lower acidities and was much less frequently followed by further acid production. The development of unpalatability was normally detected after the samples had become unstable to alcohol.

Storage at 6° C.

The development of titratable acidity in pasteurized milks held in a refrigerator at 6° C. is shown in Figs. 3 and 4 and the time interval before instability to alcohol and boiling and the type of deterioration is given in Tables 9 and 10.

Table 7. *The time (days) taken to produce certain changes in* H.T.S.T. *pasteurized milk during storage at* 18·5° C. *as shown by titratable acidity, precipitation with alcohol and clot-on-boiling*

Sample no.	Titratable acidity: Onset of increase	Titratable acidity: 1·8	Titratable acidity: 2·8 or over	Alcohol grade: II	Alcohol grade: III	Clot-on-boiling	Type of deterioration
56	6	6	6½	6	—	6	Very slow acid production
57	3	3	4	3	4	3	Sweet clot, later acid and cheesy
58	2½	2½	3	2½	3	3	Sweet clot, then rapid increase in T.A. and acid clot
59	3	3	4	3	4	3	Sweet clot, later acid clot (alc.) and putrid (4 days)
60	3	3	>3	3	—	3	Sweet clot, later putrid (4 days)
61	2	3	3	3	3	3	Acid clot, bitter
62	2½	3	4	2½	—	3	Sweet clot, later acid clot
63	3	4	>4	3	—	4	Sweet clot, slightly cheesy
64	2	3	3	3	3	3	Acid clot
65	2	2	2½	2	2½	2½	Sweet clot, later acid clot
66	2½	4	>4	4	—	4	Sweet clot, slightly cheesy
68	2	2½	>2½	2½	—	2½	Sweet clot (T.A. 2·4)
69	2½	3	3	2½	3	3	Sweet clot, then rapid acid production and C.O.B., bitter
70	2	2	2½	2	2½	2½	Sweet clot (T.A. 2·1), later acid clot
71	2	2½	2½	2½	2½	2½	Acid clot
73	2	2	2½	2	2½	2½	Sweet clot, later acid clot and viscous
74	2	3	>3	3	—	3	Sweet clot (T.A. 2·1)
75	2½	2½	3	2½	3	3	Sweet clot (T.A. 2·0), then rapid acid production and C.O.B.
76	3½	3½	>3½	3½	—	3½	Sweet clot and bitter
77	2½	2½	3	2½	3	2½	Sweet clot (T.A. 1·9), then rapid acid production and C.O.B.
78	3	3	3½	3	3½	3	Sweet clot (T.A. 2·1), later acid clot (alc.)
79	2	4	4	3½	4	4	Sweet clot, later acid clot and bitter
80	2	3½	>3½	3	—	3½	Sweet clot
81	3	3	3	3	3	3	Acid clot

T.A., titratable acidity; C.O.B., clot-on-boiling.

As with milks stored at 18·5° C. acid production frequently showed a sudden sharp increase after periods varying from 2 to 7 weeks in which the acidity had shown little change or only a slight and gradual increase. Contrary to the results at 18·5° C. most samples showed this sudden increase in activity and this was also true of many of the samples of L.T. pasteurized milk. With L.T. pasteurized milk, as with the same milks at 18·5° C., this sudden increase in acidity occurred much later than with the H.T.S.T. pasteurized milks.

All samples of H.T.S.T. pasteurized milk held at 6° C. gave a titratable acidity of 1·8 in times varying from 3 to 10 weeks but thirteen of the twenty-four samples failed to reach an acidity of 2·8. Of the L.T. pasteurized milks eight of the twenty-two samples examined failed to reach a titratable acidity of 1·8 even after 9–13 weeks but half of the samples did eventually develop an acidity of 2·8 and gave clot-on-boiling due to acidity.

Table 8. *The time (days) taken to produce certain changes in* L.T. *(laboratory) pasteurized milk during the storage at* 18·5° C. *as shown by titratable acidity, precipitation with alcohol and clot-on-boiling*

Sample no.	Titratable acidity: Onset of increase	Titratable acidity: 1·8	Titratable acidity: 2·8 or over	Alcohol grade II	Clot-on-boiling	Type of deterioration
56	3	3	>6½	3½	6½	Sweet clot, slow acid production to C.O.B.
57	2½	2½	>4	2½	2½	Sweet clot, later bitter
58	2½	3	4	3	3	Sweet clot
59	2½	3	4	2½	2½	Sweet clot, later putrid (4 days)
60	3	3	4	3	4	Sweet clot, later cheesy and acid C.O.B.
61	3	3½	>3½	3½	3½	Sweet clot
62	2½	3	>4	3	3	Sweet clot
63	3	4	>4	4	4	Sweet clot (cheesy)
64	3	3	>3	3	3	Sweet clot
65	3	3	>3	3	3	Sweet clot
66	3	4	>4	3	3	Sweet clot, later slightly bitter
68	2½	>2½	>2½	2½	2½	Sweet clot
69	3	3½	>3½	3½	3½	Sweet clot
70	3	>3½	>3½	3½	3½	Sweet clot, T.A. below 1·8
71	2½	3½	>3½	3½	3½	Sweet clot
73	3	>3	>3	3	3	Sweet clot, T.A. below 1·8
74	2	3	>3	3	3	Sweet clot
75	3	3	3	3	3	Acid clot, sour
76	3	3	>3	3	3	Sweet clot
77	3	>3	>3	3	3	Sweet clot, T.A. below 1·8
78	3½	4	>4	3	4	Sweet clot
79	3	3	>3½	3½	3½	Sweet clot
80	3	4	4	3	3	Sweet clot, later sharp rise in T.A. (3½–4 days)
81	3	3	>3	3	3	Sweet clot

T.A., titratable acidity; C.O.B., clot-on-boiling.

Table 9. *The time (weeks) taken to produce certain changes in* H.T.S.T. *pasteurized milk during storage in the refrigerator* (6° C.) *as shown by titratable acidity, precipitation with alcohol and clot-on-boiling*

Sample no.	Titratable acidity: Onset of increase	Titratable acidity: 1·8	Titratable acidity: 2·8 or over	Alcohol grade II	Clot-on-boiling	Type of deterioration
56	5	5	>10	6	>10	Sweet clot (T.A. 2·3) after 6 weeks
57	7	7	>10	—	>10	Slow increase in acidity to 2·0, bitter
58	3	3	5	3	5	Sweet clot, later acid C.O.B.
59	4	4	>7	5	5	Slow acid production, bitter
60	9	10	>13	—	>13	No C.O.B. viscous, bitter
61	4	6	>9	6	7	Gradual increase in acidity to 2·3, fishy
62	3	3	8	—	8	Slow acid production
63	6	7	>9	7	9	Sweet clot, later C.O.B. at T.A. 2·0
64	7	7	>8	7	8	Sweet clot, later C.O.B. at T.A. 2·3
65	6	6	>9	6	>9	Sweet clot, later bitter
66	6	6	>8	7	8	Sweet clot (T.A. 2·0), slightly bitter, later yeasty
68	6	7	9	7	9	Sweet clot (T.A. 2·4), bitter, later acid C.O.B.
69	5	5	6	5	6	Sweet clot (T.A. 2·2), later acid C.O.B.
70	5	5	5	5	5	Acid clot
71	4	4	5	4	5	Sweet clot (T.A. 2·4), later acid C.O.B.
73	2	3	>3	3	3	Acid clot, bitter-cheesy
74	5	6	9	6	9	Sweet clot (T.A. 2·3), bitter, later acid C.O.B., cheesy
75	4	4	4	4	4	Acid clot, bitter
76	3	4	>4	4	4	Sweet clot (T.A. 2·0)
77	4	4	5	4	5	Sweet clot (T.A. 2·0), later acid C.O.B.
78	8	8	>8	8	8	Acid clot
79	4	4	>6	—	5	Sweet clot (T.A. 2·1), bitter
80	3	6	6	6	6	Slow acid production, acid clot
81	5	5	7	5	7	Sweet clot, later acid C.O.B.

T.A., titratable acidity; C.O.B., clot-on-boiling.

Table 10. *The time (weeks) taken to produce certain changes in* L.T. *pasteurized milk during storage in the refrigerator* (6° C.) *as shown by titratable acidity, precipitation with alcohol and clot-on-boiling*

Sample no.	Titratable acidity: Onset of increase	Titratable acidity: 1·8	Titratable acidity: 2·8 or over	Alcohol grade II	Clot-on-boiling	Type of deterioration
56	>10	>10	>10	—	>10	No change in 10 weeks
57	10	>11	>11	—	>11	Little change in 11 weeks
58	3	5	>7	4	7	Sweet clot, later acid C.O.B.
59	5	5	11	5	11	Sweet clot, later acid C.O.B., cheesy
60	6	6	10	7	10	Sweet clot, later acid C.O.B., cheesy
61	4	7	9	7	9	Slow acid production, bitter, later sour
62	7	8	12	8	12	Sweet clot, bitter, delayed acidity to C.O.B.
63	8	>9	>9	>9	>9	Little change in 9 weeks
64	Not examined					
65	7	>11	13	12	13	Delayed acid production, C.O.B. after 11 weeks
66	6	8	10	8	10	Sweet clot, later acid C.O.B. and fishy
68	6	>13	>13	14	>14	Sweet clot after 14 weeks
69	5	7	9	7	9	Sweet clot (7 weeks), bitter, later acid C.O.B.
70	6	6	8	7	9	Sweet clot (T.A. 2·2), later acid C.O.B.
71	5	>11	>14	14	>14	Sweet clot after 14 weeks, cheesy
73	3	7	>12	7	>12	Sweet clot, bitter, lumpy (12 weeks)
74	6	6	9	6	9	Sweet clot, later acid C.O.B. and bitter
75	6	6	6	6	6	Rapid acid production after 4 weeks, acid C.O.B. (6 weeks)
76	6	6	6	6	6	Rapid acid production after 4 weeks, acid C.O.B. (6 weeks)
77	7	9	>13	10	>13	Sweet clot (T.A. 2·3), acidity lower 11–13 weeks, 'bitty' cream
78	Not examined					
79	9	>13	>13	—	>13	Little change in 13 weeks, cardboardy
80	3	7	>7	7	7	Slow acid production to C.O.B. (7 weeks), bitter
81	>12	>12	>12	—	>12	Little change in 12 weeks, 'bitty' cream

T.A., titratable acidity; C.O.B., clot-on-boiling.

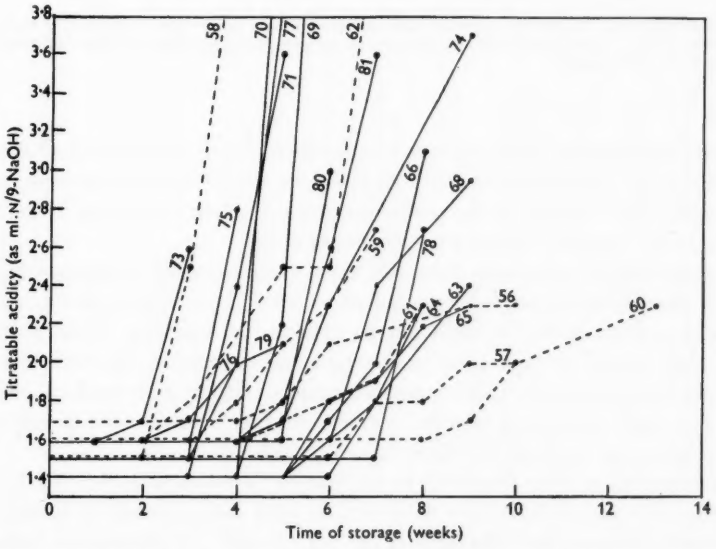

Fig. 3. The development of titratable acidity in twenty-four samples of H.T.S.T. pasteurized milk during storage in the refrigerator (6° C.). Numbers: code no. of sample. Broken lines: samples in which the predominating microflora was determined.

The mean keeping quality as indicated by the alcohol test for the fifteen samples for which data are available for both the H.T.S.T. and L.T. pasteurized milks was 5 and 8 weeks respectively. A definite end-point was not always obtained for the clot-on-boiling test, so that mean values for the keeping quality assessed on this basis would be misleading, especially as the times varied considerably from sample to sample. Reference to Tables 9 and 10 shows, however, that samples of L.T. pasteurized milk took much longer than the H.T.S.T. pasteurized milk to reach the point of instability to boiling, and that many of the L.T. pasteurized milks had not reached this point after 10–14 weeks at 6° C.

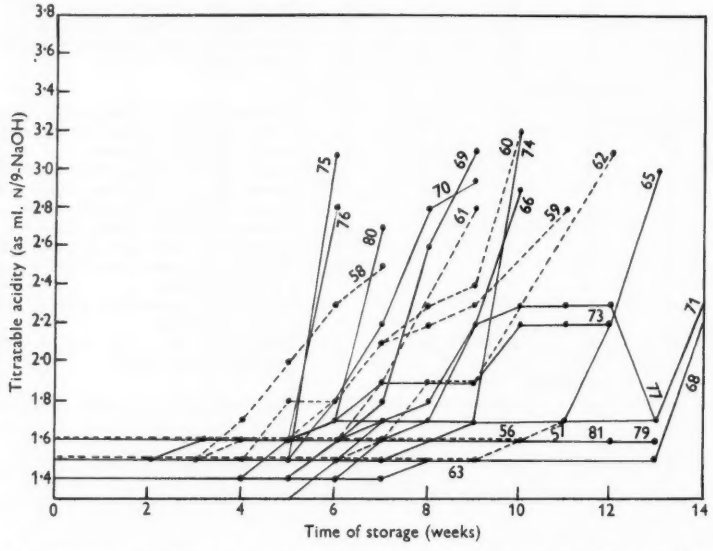

Fig. 4. The development of acidity in twenty-two samples of L.T. pasteurized milk during storage in the refrigerator (6° C.). Numbers: code no. of sample. Broken lines: samples in which the predominating microflora was determined.

Microflora

The predominating microflora in seven samples (nos. 56–62) was determined at intervals during storage at 18·5 and 6° C. for both the H.T.S.T. and the corresponding L.T. pasteurized milks. The changes in the predominating microflora are shown in Table 11 for storage at 18·5° C. and in Table 12 for storage at 6° C.

In the examination of the microflora only those species of micro-organisms which were capable of developing on plates incubated at 22 or 6° C. were determined, since those which failed to grow at 22° C. could not be expected to multiply in the stored milk, although they might be capable of producing some changes in the milk in the early stages of the incubation period. All samples contained initially a preponderance of micrococci, *Alcaligenes* sp. and aerobic spore-forming bacilli.

The predominating microflora will be considered in relation to the development of titratable acidity and of instability to alcohol at both temperatures of incubation, and the differences between the behaviour of the H.T.S.T. and L.T. pasteurized milks will be considered for the individual samples.

Table 11. *The predominating species of micro-organisms in* H.T.S.T. *and the corresponding* L.T. *pasteurized milk during storage at* 18·5° C.

No. of sample	Type of milk	Time of storage at 18·5° C. (days)							
		1	1½	2	2½	3	3½	4	6½
56	H.T.S.T.								
	L.T.								
57	H.T.S.T.								
	L.T.								
58	H.T.S.T.								
	L.T.								
59	H.T.S.T.								
	L.T.								
60	H.T.S.T.								
	L.T.								
61	H.T.S.T.								
	L.T.								
62	H.T.S.T.								
	L.T.								

Table 11. *The predominating species of micro-organisms in* H.T.S.T. *and the corresponding* L.T. *pasteurized milk during storage at* 18·5° C.

No. of sample	Type of milk	Time of storage at 18·5° C. (days) 1	1½	2	2½	3	3½	4	6½	7
56	H	*Alcaligenes* *Ps. fluorescens*	*Alcaligenes* *Ps. fluorescens*	*Alcaligenes*	*Alcaligenes*	*Alcaligenes*	*Alcaligenes*	*Alcaligenes*	*Alcaligenes* *Achromobact.* *Str. lactis*	*Str. lactis* *Alcaligenes* *Achromobact.*
	L	*Alcaligenes*	—	*Alcaligenes*	*Alcaligenes* *Ps. fluorescens*	*Alcaligenes* *Pseudomonas*	*Alcaligenes* *Pseudomonas*	*Alcaligenes*		
57	H	—	*Microbact.*	—	*B. subtilis* (I) *Str. lactis*	*B. subtilis* (I) *Str. lactis*	*Str. lactis* *B. subtilis* (I) *B. subtilis* (II)	*Str. lactis* *B. subtilis* (II)	(5½ days) *Str. lactis*	
	L	Micrococci	*Alcaligenes*	—	*B. subtilis* (I) *B. subtilis* (II)	*Alcaligenes*	—	*Alcaligenes* *B. subtilis* (II)	*Str. lactis* *Alcaligenes* *B. subtilis* (II)	
58	H	*Alcaligenes* *Ps. fluorescens* *Pseudomonas* *Bact. aerogenes*	—	*Alcaligenes* *Str. lactis* *Pseudomonas*	*Alcaligenes* *Str. lactis* *Pseudomonas* *Bact. aerogenes*	*Str. lactis* *Alcaligenes*				
	L	—	—	*Alcaligenes* *Pseudomonas*	*B. subtilis* (I) *B. subtilis* (II)	*B. subtilis* (I) *B. subtilis* (II) *Pseudomonas*	*B. subtilis* (I) *B. subtilis* (II) *Pseudomonas*	*B. subtilis* (I) *Pseudomonas*		
59	H	*Pseudomonas*	*Pseudomonas* *Ps. fluorescens*	*Pseudomonas* *Alcaligenes*	*B. subtilis* (I) *Pseudomonas*	*B. subtilis* (I) *Pseudomonas*	—	*Str. lactis* *Pseudomonas* *B. subtilis* (I)		
	L	*Alcaligenes* *Bacillus* *Pseudomonas*	*Alcaligenes* *B. pumilus* *Pseudomonas*	*Alcaligenes* *Pseudomonas*	*Alcaligenes* *Pseudomonas* *Bacillus*	*Pseudomonas*	—	*Bact. aerogenes* *Pseudomonas*		
60	H	*Microbact.*	*B. subtilis* (I)	*B. subtilis* (I) *Ps. fluorescens*	*Ps. fluorescens*	*Ps. fluorescens* *Alcaligenes*	*Ps. fluorescens* *B. subtilis* (I)	*Ps. fluorescens*		
	L	*Microbact.*	*Microbact.*	*B. subtilis* (I) *Pseudomonas* *Ps. fluorescens*	*Pseudomonas* *B. subtilis* (I)	*Pseudomonas* *Ps. fluorescens*	*Pseudomonas* *Ps. flourescens* *Str. lactis*	*Pseudomonas* *Str. lactis* *Ps. fluorescens*		
61	H	*Alcaligenes* *Microbact.*	*Microbact.* *Alcaligenes*	*Alcaligenes* *Pseudomonas*	—	*Str. lactis* *Alcaligenes* *Pseudomonas*				
	L	*Microbact.* *Str. faecalis*	*Microbact.* *Str. faecalis* *Alcaligenes*	*B. cereus*	—	*B. cereus* *Flavobact.*	*B. cereus* *Bacillus*	*Bacillus*		
62	H	*Microbact.* *Alcaligenes* *B. cereus*	*Alcaligenes* *B. cereus*	*Alcaligenes* *Str. kefir* *B. cereus*	*Alcaligenes* *Str. kefir*	*Alcaligenes* *Str. kefir*	—	*Alcaligenes* *Str. kefir*		
	L	*Microbact.* *Bacillus*	*Microbact.* *B. subtilis* (I)	*Alcaligenes* *Pseudomonas* *Bacillus*	*Alcaligenes* *Pseudomonas* *B. subtilis* (I)	*Alcaligenes* *Pseudomonas*	—	*Alcaligenes* *Pseudomonas* *Bact. coli*, type I		

Table 12. *The predominating species of micro-organisms in* H.T.S.T. *and the corresponding* L.T. *pasteurized milk during storage at* 6° C.

No. of sample	Type of milk	Time of storage at 60° C. (weeks) 2	3	4	5	6	7	8	9	10
56	H	*Microbact.* Yeast *Achromobact.*	Yeast	Yeast *Achromobact.*	Yeast	Yeast	Yeast	Yeast		
	L	*Microbact.*	*Microbact.* *Bacillus*	*Microbact.* *Bacillus*	*Microbact.* *Alcaligenes* *Achromobact.*	*B. circulans*	—	—	Yeast	
57	H	Yeast	Yeast	Yeast	Yeast	Yeast	Yeast	Yeast	Yeast	Yeast
	L	*Microbact.*	*Microbact.* Micrococci	*Achromobact.*	*Achromobact.*	—	*Achromobact.* *Microbact.*	*Achromobact.* *B. circulans*	*Achromobact.* *B. circulans*	*Str. lactis* *B. circulans*
58	H	*Ps. fluorescens* *Pseudomonas*	—	—	*Ps. fluorescens* *Str. lactis* *Pseudomonas*					
	L	*Ps. fluorescens* *Pseudomonas*	*Pseudomonas*	—	*Ps. fluorescens* *Pseudomonas*	*Ps. fluorescens*	*Ps. fluorescens*			
59	H	*Ps. fluorescens*	—	*Ps. fluorescens*	*Ps. fluorescens*	*Ps. fluorescens*	*Ps. fluorescens*			
	L	*Pseudomonas*	—	*Pseudomonas*	*Pseudomonas*	*Pseudomonas*	*Pseudomonas*	*Pseudomonas*	*Pseudomonas* *Flavobact.* *B. circulans*	*Pseudomonas*
60	H	*Microbact.*	*Microbact.* *Flavobact.* Yeast	*Flavobact.* Yeast	—	*Flavobact.*	*Flavobact.*	*Flavobact.*	*Flavobact.*	*Flavobact.*
	L	*Microbact.* *Alcaligenes* *Pseudomonas*	*Pseudomonas* *Alcaligenes*	*Pseudomonas* *Alcaligenes*	—	*Pseudomonas*	*Pseudomonas*	—	*Pseudomonas*	*Ps. fluorescens* *Pseudomonas* *Alcaligenes*
61	H	*Ps. fluorescens*	*Ps. fluorescens*	*Ps. fluorescens*	—	*Ps. fluorescens*	*Ps. fluorescens*			
	L	*Microbact.* *Flavobact.* *Ps. fluorescens*	*Microbact.*	*B. circulans* *Microbact.*	—	*B. circulans* *Microbact.*	*B. circulans*	*B. circulans*	*B. circulans* *Str. lactis*	
62	H	*Pseudomonas*	*Pseudomonas*	—	*Pseudomonas*	*Pseudomonas*				
	L	—	*Microbact.* *Pseudomonas* *Flavobact.*	—	*Pseudomonas*	*Pseudomonas*	—	*Pseudomonas*	—	*Pseudomonas*

Sample 56. In the H.T.S.T. pasteurized milk held at 18·5° C. the microbacteria and micrococci were overgrown after 1 day by an alkali-producing *Alcaligenes* sp. and by *Pseudomonas fluorescens* which produces proteolytic changes in milk. Later *Alcaligenes* sp. predominated, but after 6½–7 days *Str. lactis* and an acid-producing *Achromobacterium* sp. were superseding the alkali producer. The failure of the milk to become alkaline in the first 6 days (Fig. 1) in spite of the predominance of *Alcaligenes* sp. suggested that slow growth of the acid-producing species, especially *Str. lactis*, was taking place but was not detectable by the methods of examination used. A similar flora was observed in the corresponding L.T. pasteurized milk, and the gradual increase in titratable acidity (Fig. 2) may have been due to the slow growth of *Str. lactis*, but this could not be confirmed owing to the lack of data after storage for 6½–7 days.

In the L.T. pasteurized milk *Alcaligenes* sp. did not entirely outgrow *Ps. fluorescens* or a non-proteolytic non-acid-producing strain of *Pseudomonas*, and the instability to alcohol developed after storage for 3½ days (Table 8) may have been due to the *Pseudomonas* sp. since it was not observed in the H.T.S.T. pasteurized milk until the titratable acidity was 2·8.

When the same milks were stored at 6° C. an alkali-producing yeast overgrew the initially dominant microbacteria in 3 weeks in the H.T.S.T. but not until after 9 weeks in the L.T. pasteurized milk. The types of bacteria isolated from the L.T. pasteurized milk suggested that these were survivors of those initially present, and that growth of other species was extremely slow at 6° C.

Sample 57. In the H.T.S.T. pasteurized milk at 18·5° C. microbacteria were still dominant after 1½ days, but in 2½ days they had been superseded by *Bacillus subtilis* and smaller numbers of *Str. lactis*. On longer storage *Str. lactis* gradually increased in numbers relative to *B. subtilis* in which an acid-producing variant (designated II) gradually became the main type of spore-former. The gradual increase in titratable acidity at 18·5° C. which began after 2½ days (Fig. 1) was presumably due to *Str. lactis* assisted by the acid-producing variant of *B. subtilis* (II). The sweet clot obtained after storage for 3 days (Table 7) was probably due to the non-acid producing proteolytic strain of *B. subtilis* (I). Similar changes in microflora were observed in the L.T. pasteurized milk, although *Alcaligenes* sp. were also isolated and *Str. lactis* was not detected until after storage for 5½ days.

As with sample 56 growth in the L.T. pasteurized milk at 6° C. appeared to be very slow, but the detection of *Str. lactis* after 10 days was correlated with the slight increase in titratable acidity which was observed at this time. In the H.T.S.T. pasteurized milk yeasts rapidly outgrew other species, and no explanation was obtained of the increase in titratable acidity which took place after 6 weeks, but it is possible that this may have been due to *Str. lactis*.

Sample 58. At 18·5° C. the H.T.S.T. pasteurized milk began to show an increase in titratable acidity in 2 days and a sharp increase in 2½ days (Fig. 1). This coincided with the appearance after 2 days of *Str. lactis* which became the dominant species after 3 days when the milk clotted on boiling. The instability to alcohol shown after 2½ days may have been due at least in part to the presence of *Pseudomonas* sp. This species assisted by the growth of *B. subtilis* (I) may have been responsible for the sweet clot in the L.T. pasteurized milk. This milk differed from the H.T.S.T. pasteurized milk in developing a preponderance of *B. subtilis* (both variants), and the more gradual development and lower titratable

acidity may have been due to slow growth of small numbers of *Str. lactis* although the streptococcus was not detected.

At 6° C. both H.T.S.T. and L.T. pasteurized milks showed rapid development of *Ps. fluorescens* and smaller numbers of several other *Pseudomonas* sp. associated with instability to alcohol after 3 and 4 weeks respectively. The titratable acidity began to increase after 2 and 3 weeks, and increased gradually to produce an acid clot on boiling. *Str. lactis* was detected at the time of clot-on-boiling in the H.T.S.T. but not in the L.T. pasteurized milk.

Sample 59. The proteolytic strain of *B. subtilis* and a *Pseudomonas* sp., which was a weak acid-producer, predominated at 18·5° C. in the H.T.S.T. pasteurized milks. In the L.T. pasteurized milks the *Alcaligenes* sp., with a few spore-forming bacilli, were gradually replaced by the *Pseudomonas* sp. Both milks had undergone putrefaction and became unstable to alcohol and to heat after 2½–3 days. Both milks showed a gradual increase in acidity to which the *Pseudomonas* sp. may have contributed, but which was more likely to have been due to subdominant numbers of *Str. lactis* in the H.T.S.T. and of *Bact. aerogenes* in the L.T. pasteurized milks since these bacteria eventually became predominant after 4 days at 18·5° C. The higher maximum acid production in the L.T. than in the H.T.S.T. pasteurized milk may have been due to the difference in the dominant acid-producing species.

At 6° C. *Ps. fluorescens* in the H.T.S.T. and the weak acid-producing *Pseudomonas* sp. in the L.T. pasteurized milks were the dominant species and were probably responsible for the instability to alcohol which developed after 5 weeks. The increase in titratable acidity, which began after 3 and 5 weeks respectively, was possibly due to subdominant micro-organisms which were not detected.

Sample 60. Changes in the dominant microflora at 18·5° C. were evident after 1½ days in the H.T.S.T. and after 2 days in the L.T. pasteurized milk, and in both milks were due to the growth of the proteolytic species, *B. subtilis* and *Ps. fluorescens*, with the weak acid-producing *Pseudomonas* sp. more frequent in the L.T. pasteurized milk. The L.T. pasteurized milk also differed from the H.T.S.T. product in showing a sudden rapid increase in titratable acidity after 3½ days, and this was associated with the detection of *Str. lactis*.

At 6° C. the initial microflora of the H.T.S.T. pasteurized milk was gradually overgrown by *Flavobacterium* sp., mainly *F. lactis*, which gave a viscous consistency to the milk but failed to produce sufficient acid to give a clot on boiling, even after storage for 13 weeks. In the L.T. pasteurized milk the same *Pseudomonas* sp. developed at 6° C. as at 18·5° C., and the micro-organism responsible for the relatively sharp increase in the titratable acidity after 5 weeks was not detected.

Sample 61. The isolation of microbacteria after storage at 18·5° C. for 1½ days suggested slow growth in both types of pasteurized milks. In the H.T.S.T. pasteurized milk the *Alcaligenes* sp. and the *Pseudomonas* sp. (weak acid-producer) gradually replaced the microbacteria. After 3 days *Str. lactis* was also present, and the appearance of this species was reflected in the rapid increase in titratable acidity which began after 2½ days. In the L.T. pasteurized milk acid production slowly increased from 2½ to 4 days, and the instability to alcohol or to boiling at 3 days was presumably due to *B. cereus* and other spore-forming bacilli. *Ps. fluorescens* predominated in the H.T.S.T. pasteurized milk held at 6° C., and the gradual increase in titratable acid after storage for 4 weeks must be attributed to some undetected subdominant species. In the L.T. pasteurized milk no

Pseudomonas spp. were detected, and the microbacteria were only slowly overgrown by *B. circulans* which, with *Str. lactis*, was responsible for the increase in titratable acidity observed after 6 weeks.

Sample 62. In the H.T.S.T. pasteurized milk held at 18·5° C. the *Alcaligenes* sp. gradually became predominant and was associated for the first 2 days with *B. cereus* and from the second day with *Str. kefir*. The alkali produced by the *Alcaligenes* sp. was not sufficient to prevent an increase in titratable acidity due to *Str. kefir*. In the L.T. pasteurized milk also the *Alcaligenes* sp. gradually became predominant and was associated with the weak acid-producing *Pseudomonas* sp. and with smaller numbers of spore-forming rods. These species, especially the *Bacillus* sp., were presumably responsible for the instability to alcohol and to boiling which developed after storage for 3 days. The detection of *Bact. coli* type I after 4 days suggested that this bacterium may have contributed to the increase in titratable acidity which took place after storage for $2\frac{1}{2}$ days.

At 6° C. *Pseudomonas* sp. were the only bacteria isolated from both the H.T.S.T. and the L.T. pasteurized milks. The development of the titratable acidity was more rapid and reached a higher level in the H.T.S.T. than in the L.T. pasteurized milk. This difference may have been due to the variation in the strains of *Pseudomonas* sp. isolated from the H.T.S.T. and the L.T. pasteurized milks. However, after consideration of the high acidity (3·8) produced in the H.T.S.T. pasteurized milk and of the results obtained with other samples, the possibility that acid was also produced by undetected subdominant species, such as streptococci, cannot be entirely overlooked.

In another series of twenty H.T.S.T. pasteurized milks the microflora was determined during storage at 6° C. but was not correlated with the titratable acidity or instability to alcohol. Deterioration was due to *Pseudomonas* spp. (frequently *fluorescens*) in nine milks, to *Str. kefir* in six samples, in two of which it was associated with *Pseudomonas* spp. and in five other samples to *Bact. aerogenes* type I, *B. circulans*, *Alcaligenes* sp. plus a yeast, and to a yeast only respectively in five other samples. Nearly all milks containing a preponderance of *Pseudomonas* sp. developed a bitter taste. The sample in which *Bact. aerogenes* became dominant had given a positive presumptive coliform test in 0·1 ml. of the fresh pasteurized milk. The only other sample giving, before storage, a positive coliform test in 0·1 ml. gave an acid clot in 2 weeks which may have been due to growth of coliform bacteria and was certainly not due to the *Pseudomonas* sp. which was the only species detected on the Yeastrel milk agar plates.

DISCUSSION

The development of titratable acidity in commercial H.T.S.T. pasteurized milk taken direct from the pasteurizer, and in laboratory L.T. pasteurized milk when held at either 18·5 or 6° C., has been shown to vary considerably in the time at which acid production began to increase and in the rate at which it was produced. Some pasteurized milks showed little change or a gradual increase in titratable acidity, and then a sudden rapid increase which might occur after either short or long periods of storage, others showed only a gradual increase to a lower maximum, and a few showed little change in titratable acidity even after prolonged storage.

The study of a limited number of samples showed that although the predominating bacteria consisted of a relatively small number of different species there was a fairly

wide variation in both the type and the rate of deterioration in the pasteurized milks during storage.

The overgrowth of the initial microflora by alkali-producing and proteolytic bacteria which has been noted by earlier workers was shown in the samples examined in the present study, but in only one instance, after prolonged storage, did this change in the predominating flora cause an actual reduction in titratable acid, and only rarely did it reduce the rate of acid production. It seems probable that in most milks there are small numbers of acid-producing bacteria present which neutralize the effect of the numerically predominant alkali-producers. These acid-producing bacteria frequently became predominant on prolonged storage and, as previously shown by Rowlands & Provan (1), were finally responsible for the production of sufficient acid to clot the milk on boiling. Members of the *Str. lactis* group were found by Rowlands & Provan to produce an acid clot after 3 days at 15·5° C. Ashton (4) failed to isolate these streptococci after 2 days at 18° C., but the results of the present investigation suggested that these streptococci may have been present in small numbers and might have been detected in Ashton's samples after longer incubation. In the samples which were examined in detail *Str. lactis* was detected in six of the seven samples in either the H.T.S.T. or the L.T. pasteurized milk or in both types of milk, and *Str. kefir* in the seventh sample. *Str. lactis* and *Str. kefir* were also detected in pasteurized milks held at 6° C. The appearance of *Str. lactis* and of *Str. kefir* was associated with the rapid increase in the rate of acid production. It appears that small numbers of these streptococci survived pasteurization and grew in the milk even at low temperatures, and that given a long enough incubation time they would ultimately overgrow the other bacteria present. The marked variation in the time at which rapid acid production occurred probably depends mainly on the number of streptococci which survive pasteurization, although it may also be influenced by the associated microflora. Abd-el-Malek & Gibson (13) have found that in pure culture a few cells of *Str. lactis* may on occasion survive heating at 63° C. for 30 min. and the *Str. kefir* frequently survives this heat treatment and not uncommonly predominates in milk held at 10–22° C.

Many samples of pasteurized milk showed instability to alcohol or less frequently to heat at low or moderate (less than 2·8) titratable acidity, and as Ashton (4) has pointed out, this was due mainly if not entirely to the alkali-producing and proteolytic bacteria rather than the acid producers. These bacteria were also responsible for putrefactive changes and bitter taints in the milk.

Most of the H.T.S.T. pasteurized milks showed little change in titratable acidity in the first 2 days at 18·5° C. or in 2 weeks at 6° C., and in the L.T. pasteurized milks there was usually little change in $2\frac{1}{2}$ or even 3 days at 18·5° C. or in 4 weeks at 6° C. The slower rate of deterioration in L.T. than in H.T.S.T. pasteurized milks has already been noted by Rowlands & Provan (1) and by Ashton (4). This was shown in the present study not only in the development of titratable acidity but also in the longer period of storage during which microbacteria were among the predominating bacteria in the L.T. pasteurized milk. Although there was little change in titratable acidity after short periods at 18·5 or 6° C. the change in the predominating microflora, as after 1–2 days at 18·5° C., suggested that the composition of the milk would alter during this period, although these changes were not usually sufficient to produce instability to 68% alcohol or to heat.

The detailed study of the microflora of the seven samples of H.T.S.T. and L.T. pasteurized milks has shown that the identification of the predominating microflora failed to provide sufficient information to explain the changes in acid production which occurred in such milks during storage. Some method of detecting the subdominant groups of bacteria is essential if an accurate picture of the microbial population is to be obtained.

SUMMARY

1. The numbers and species of micro-organisms surviving in commercial high-temperature short-time pasteurized milks sampled direct from the cooling section of the pasteurizer have been determined and compared with those in the corresponding raw milk and in the raw milk after low-temperature pasteurization in the laboratory.

2. The geometric mean counts at 37° C. for twenty-five samples of raw, L.T. and H.T.S.T. pasteurized milks were 74,130, 1,740 and 3,200 per ml. respectively. The corresponding figures at 30° C. were 123,030, 7,080 and 8,510 per ml.

3. The percentage destruction of the bacteria was slightly smaller at all four plants for the H.T.S.T. pasteurized milks than for the corresponding L.T. pasteurized milks. The mean percentage destruction for the L.T. pasteurized milks was 97·7% for bacteria growing at 37° C. and 94·3% for bacteria growing at 30° C. The corresponding figures for the H.T.S.T. pasteurized milks were 95·9 and 93·1%.

4. The keeping quality (clot-on-boiling) did not differ by more than half a day (one reading) for fifteen (62·5%) of the twenty-four samples of H.T.S.T. and L.T. pasteurized milks. Eleven samples had a longer and six samples a shorter keeping quality (clot-on-boiling) after L.T. than after H.T.S.T. pasteurization.

5. Following pasteurization there was a decrease in the numbers of alkali-producing and peptonizing bacteria, and a corresponding increase in the acid-producing bacteria which constituted the major portion of the microflora of the pasteurized milks. Many of these bacteria produced only moderate amounts of acid.

6. Pasteurization by either the L.T. or H.T.S.T. method caused a marked decrease in the proportion of the streptococci and non-sporing rods and an increase in the proportion of microbacteria and micrococci.

7. Streptococci of the *lactis-cremoris* group were present in all but one of the raw milks, most of which also contained *Str. faecalis*. No streptococci were detected in about half the pasteurized milks but from the remainder *Str. thermophilus* and occasionally *Str. faecalis* were isolated. Microbacteria were isolated from all but one of the pasteurized milks, and were also detected in small numbers in some of the raw milks.

8. In another series of H.T.S.T. and the corresponding L.T. pasteurized milks the changes in titratable acidity and in stability to alcohol and to boiling were determined at intervals during storage at 18·5° C. and in a refrigerator at 6° C.

9. In H.T.S.T. pasteurized milks stored at 18·5° C. about half the samples showed a sudden rapid increase in titratable acidity after 2–2½ days, while the remainder produced only a gradual increase in acidity. At 6° C. both L.T. and H.T.S.T. pasteurized milks frequently showed a sudden increase in acidity following a period of at least 2 weeks in which the acidity showed little change or only a gradual increase. At both temperatures the acid production was slower in the L.T. than in the H.T.S.T. pasteurized milks.

10. Deterioration in the keeping quality of pasteurized milk during storage was

associated more frequently with changes leading to instability to alcohol or to boiling than with acid production.

11. The predominating species of micro-organisms in seven samples of H.T.S.T. pasteurized milks were determined at intervals during storage at 18·5 and 6° C., and the changes in the microflora considered in relation to the changes observed in the titratable acidity and in the stability to alcohol and to boiling.

12. During storage at 18·5° C. the predominating flora of the freshly pasteurized milk was rapidly replaced by an alkali-producing species of *Alcaligenes* or by *Ps. fluorescens* associated with other Pseudomonads and *B. subtilis*. Sudden rapid increase in titratable acidity was associated with the appearance of the *Str. lactis* group among the predominating bacteria. At 6° C. similar changes took place more slowly, and in some samples *B. circulans* and yeasts were the predominating micro-organisms. The changes in the predominating microflora started more slowly in L.T. than in H.T.S.T. pasteurized milk.

13. The identification of the predominating micro-organisms in pasteurized milks during storage failed to provide a complete explanation of the changes in acid production since this method failed to indicate the influence of subdominant groups of bacteria.

The authors are grateful for the helpful co-operation which was so willingly given by the managements and staffs of the four creameries from which the samples of raw and H.T.S.T. pasteurized milk were obtained.

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510. REACTIVATION OF MILK PHOSPHATASE FOLLOWING HEAT TREATMENT. I

By R. C. WRIGHT and J. TRAMER
United Dairies Research Laboratories, London

(With 1 Figure)

Continuous processes for the production of sterile milk by rapid heating to temperatures in excess of 100° C., followed by rapid cooling, have been developed during the last few years in the U.S.A. and Europe. Under appropriate conditions it is claimed that the flavour of the milk so treated is unimpaired. The work reported in this paper originated in an examination of samples of 'Uperized' milk produced by such a process in Switzerland by the Bernese Alps Company of Konolfingen.*

I. PRELIMINARY OBSERVATIONS

The samples were received in aluminium cans and were stored at various temperatures for a few weeks before carrying out a routine bacteriological and chemical examination. This examination showed that although the milks were sterile, they gave a high value on the official Kay & Graham phosphatase test(1), varying from 6·0 to >30 L.B.U. with a normal control value of <1·5 L.B.U. Further, after laboratory pasteurization a figure of <1·5 L.B.U. was obtained in each case. No evidence was obtained of the presence of any other enzymes in the milks.

Later, opportunity occurred for examination of further samples of this type of milk from the same source, and again stored cans gave high L.B.U. values though the milks were sterile. This combination of a high phosphatase test result and a normal control value with the Kay & Graham test on milk which has been sterilized by heat treatment, has not been reported before, as far as we are aware, and accordingly further investigation was carried out.

The results obtained on additional samples of this type of milk were as follows:

(1) Milk stored for 1 week or more at temperatures varying from 22 to 37° C. gave high L.B.U. values (13–28).

(2) Milk preserved with mercuric chloride at a concentration of 1 in 10,000 after processing did not give increased L.B.U. values on storage.

(3) All samples, stored or otherwise, gave normal L.B.U. values immediately after laboratory pasteurization.

(4) Samples laboratory pasteurized before storage gave high L.B.U. values on subsequent storage, but this value was less than half that given by the corresponding milk stored as received.

(5) Samples which were laboratory pasteurized after storage gave only a slightly high L.B.U. value (2·0–2·3) after subsequent storage. Pasteurization after this second storage period gave a normal L.B.U. value which did not increase on further storage.

(6) Maximum increase in L.B.U. value occurred after storage at 30° C.

* This process was developed jointly by Oursina, Ltd. and Sulzer Bros. Ltd. of Winterthur, Switzerland. The commercial development has since been vested in Alpura, Ltd. (Berne), Switzerland.

The above findings applied to milks which on arrival gave values of 2·5–2·8 L.B.U.

With other samples of this type of milk, which gave values of 1·0–1·3 L.B.U. when received, similar results were obtained except that the increase in value on storage was much smaller (2–3 L.B.U.) and that no increase was observed after laboratory pasteurization (see (4, 5)).

All samples were sterile when received as shown by routine bacteriological examination. The above facts were established from experiments in which the milks, by the use of careful aseptic technique, remained sterile during subsequent storage. Any milks contaminated during laboratory handling and storage were discarded and the results ignored.

Identification of the phosphatase

Despite the sterility of the milks giving a high phosphatase reaction, it was felt essential to prove beyond doubt that the phosphatase responsible for the high results obtained was milk phosphatase. The fact that normal values were always obtained immediately after laboratory pasteurization and that there is no published evidence of the inactivation in milk of any phosphatase of bacterial origin by laboratory pasteurization, is strong evidence that the enzyme responsible was milk phosphatase. However, the following tests were applied to milks showing a high L.B.U. value after storage:

(1) *Comparison of Kay & Graham and Aschaffenburg & Mullen tests*

The Aschaffenburg & Mullen test (A/M test) used was as modified by Tramer & Wight (2) who give comparative figures for these two tests up to 6 L.B.U.

The results obtained on the two tests with Uperized milk are given in Table 1, together with published figures for the L.B.U. values equivalent to the A/M values found with normal milk phosphatase.

Table 1

'Uperized' milk				
	L.B.U. values			Equivalent L.B.U. values for milk phosphatase (Tramer & Wight)
No. of samples	Range	Mean	A/M 2 hr. reading p.p.m. 'chlorine'	
16	0·8–1·6	1·1	N–<0·1	<1·8
5	1·6–2·3	2·0	0·1–0·15	1·8–2·3
5	2·3–3·4	2·7	0·15$^+$–0·25	2·3$^+$–3·0$^+$
5	2·7–5·1	3·9	0·3–0·3$^+$	4·0$^-$–4·0$^+$
2	6·5–7·0	6·7	0·4	6·0$^-$–6·0$^+$

Comparison of the two types of milk at higher L.B.U. values showed good agreement.

(2) *Heat resistance*

Comparison was made at 62·8° C. (145° F.), and no difference was detectable between the heat resistance of raw milk phosphatase and that developed in the stored milks as shown in Table 2.

Table 2. *A/M test 2 hr.*

Time of heating (min.)	'Uperized' milk	Pasteurized milk + 1·5% raw
0	0·75	0·5$^+$
5	0·2$^-$	0·2$^-$
7½	0·1	0·1
10	0·1$^-$	0·1$^-$
12½	Trace	Trace
15	Trace	Trace
20	Nil	Nil

(3) pH *optimum*

Since Aschaffenburg & Mullen used in their test one of the buffers given by King & Delory (3), a range of these buffers was used for preparing buffer substrate solutions of varying pH's. Again, no difference was found between raw milk phosphatase and that developed in the stored milks. Fig. 1 shows the rate of hydrolysis at pH's 10·0 and 9·3 of these two phosphatases, and also illustrates that the rate of hydrolysis is identical in both cases.

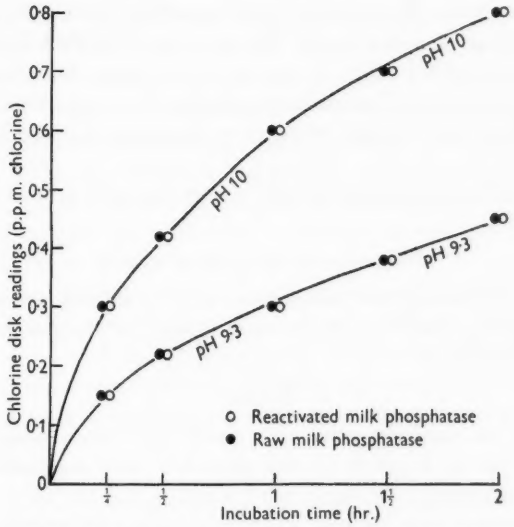

Fig. 1. Effect of pH change on the hydrolysis of A/M buffer substrate by raw milk and reactivated milk phosphatase.

Both from published work and our own experience on phosphatases of bacterial origin, a marked difference would have been expected in each of the above three tests used if the phosphatase had been of bacterial origin.

It was therefore concluded that the phosphatase developed in this type of milk on storage at suitable temperatures was identical with raw milk phosphatase.

Some experiments were carried out using both commercial pasteurized milk (H.T.S.T.) and laboratory pasteurized milk (62·8° C./35 min.), but no increase in L.B.U. value on storage at 22–37° C. was noted, except that due to the presence of large numbers of phosphatase-producing organisms.

It seemed, therefore, that whilst milk phosphatase is irreversibly inactivated by the H.T.S.T. or Holder process of pasteurization, the rapid heating of raw milk to temperatures in excess of 100° C. results in a reversible inactivation of milk phosphatase.

II. LABORATORY PRODUCTION OF MILKS GIVING POSITIVE PHOSPHATASE RESULTS ON STORAGE

At this stage of the work it was essential to find a method of rapid heating of raw milk to temperatures exceeding its boiling-point which would result in a milk giving a high phosphatase value on subsequent storage and so facilitate further investigation of this problem.

After a few preliminary experiments it was found that raw milk in sealed capillaries heated in a glycerine bath to temperatures of the order of 130° C. showed considerable phosphatase activity after a few days' storage at 30° C. Milks examined immediately after this heat treatment showed no phosphatase activity.

Technique

The capillaries used were drawn out from thin-walled glass tubing and measured approximately $11 \times 0{\cdot}1$ cm. These tubes were four-fifths filled with raw milk, leaving 1 cm. approximately at each end empty. The ends were carefully sealed and the tubes attached by means of rubber bands to one arm of a holder consisting of an L-shaped piece of glass tubing and up to four tubes per holder. The tubes were then immersed in a hot glycerine bath for a given time followed by immediate immersion in cold water for 1 min.

The volume of milk in each capillary tube was of the order of 0·05 ml.

Estimation of phosphatase activity

The problem of determining the phosphatase activity in such a small volume of milk was solved by adapting Tramer & Wight's modification of the Aschaffenburg & Mullen test in the following manner.

Method

The contents of the capillary tube were transferred into a weighed Durham tube ($2 \times \frac{1}{4}$ in.) and the tube re-weighed. To this amount of milk was added the appropriate amount of buffer substrate solution ($\times 5$ by volume) and the tube closed by a small rubber stopper. After mixing, the tube was incubated in a water-bath at 37° C. for 2 hr. The yellow colour, due to *p*-nitrophenol liberated from the substrate by phosphatase, was estimated in a Lovibond Resazurin Comparator using the disks KDA and KDAB, by comparison with a boiled milk control. To facilitate reading, the Durham tubes were placed in a small white-backed holder held in position on the ramp by means of two metal clips. In addition, the reading apertures were reduced by placing over them two smaller apertures $\frac{1}{4}$ in. in diameter, in order to restrict the field of view to the width of the Durham tubes.

For ease of handling the Durham tubes it was found convenient to place them through a hole cut in a rubber bung. This provided a stand for weighing purposes, and by reversing the position of the tube in the bung the tubes could be supported in a reductase tube rack.

Precautions

All glassware was thoroughly cleaned and the rubber stoppers washed and then boiled in distilled water before use. Owing to the fluctuations found in diameter of Durham tubes of the above size, it was found necessary to use only those selected by a suitable go-no go gauge.

Sensitivity

The use of this micro method for estimating phosphatase activity did not lead to any serious loss in sensitivity when compared with the normal procedure (1 ml. milk + 5 ml. buffer substrate solution). This will be seen from Table 3.

Table 3

% raw milk	A/M test (2 hr. reading)	
	Normal method	Micro method
2	0·8⁻	0·6
1·5	0·7⁻	0·5⁺
1·0	0·55	0·4
0·75	0·45	0·35
0·50	0·4	0·3⁺
0·40	0·35	0·25
0·30	0·3	0·2⁺
0·20	0·25	0·2⁻
0·00	<0·1	Trace

Interpretation of results

In their modification of the A/M test, Tramer & Wight used the standard Lovibond Comparator disks KDA and KDAB for measuring the yellow colour given by *p*-nitrophenol since they found that the colours concerned were readily matched. These two disks are normally used for the estimation of chlorine and are calibrated accordingly in parts per million chlorine.

Although disks are available (APTW) calibrated in μg. *p*-nitrophenol, the highest value is only 42 μg. which is equivalent to 0·4 p.p.m. chlorine and therefore, since values greater than 0·4 were sometimes obtained, the above two chlorine disks were used throughout and the degree of phosphatase activity expressed in terms of p.p.m. chlorine. It was considered that no advantage would be gained by converting these readings into μg. *p*-nitrophenol.

Before proceeding to describe in detail the work carried out using this laboratory technique of heating milk, the following terms are defined and subsequently used for the sake of brevity.

Shocked milk: raw milk heated in capillaries as described above.

Reactivated milk: milk which develops phosphatase activity after storage following heat treatment in a capillary.

Reactivation: the development of such phosphatase activity.

Experimental work on shocked milk

In order that this work should proceed with minimum delay it was essential that consideration be given to the following points:

(*a*) Time and temperature of shock treatment.
(*b*) Time and temperature of storage.
(*c*) Sterility of the shocked milk.

From some preliminary experiments it was found that these three points were all involved as far as optimal reactivation was concerned. However, since it was found that the reactivation level after 2 days was sufficient not only to be accurately measured, but also to provide ample margin for study of factors affecting reactivation, both favourably or otherwise, this time was selected and used in much of the work.

Sterility of the shocked milk could be achieved provided that the time/temperature combination was not less than 120° C. for 30 sec. Since in these preliminary experiments

it was also observed that the temperature of heating was not critical at this level, a time/temperature combination of 135° C. for 20 sec. was generally used. After cooling by immersion in cold water for 1 min. the capillaries were stored for 2 days at 30° C. A storage temperature of 30° C. was chosen since the previous work on 'Uperized' milks from Switzerland had indicated that 30° C. was probably the optimal temperature. The raw milk used was nearly always bulk raw milk from rail tanks.

These three factors, together with others, will now be considered in more detail.

Time/temperature of shock treatment

(1) *Higher temperature range*

A study has been made of the range of time and temperature within which reactivation of milk phosphatase occurs during subsequent storage. The upper limit of temperature lies between 170 and 180° C. for an immersion time of 15 sec. As the temperature of heating falls, this time can be increased, and examples of the maximum immersion time at various bath temperatures for reactivation to take place on subsequent storage are given in Table 4.

Table 4

Bath temperature (° C.)	Immersion time (sec.)
170–180	15
165	30
150	60
140	75
130	135
120	sl. >240

(2) *Lower temperature range*

Having established the fact that reactivation of milk phosphatase occurs over the heating range 120–170° C., investigation of the lower temperature range was made.

As has already been indicated, shock treatment of raw milk to temperatures below 120° C. did not bring about sterility of the milk. Therefore it was necessary to find, if possible, some means of preventing bacterial growth during the storage period, for though it is possible in our opinion to decide whether any particular phosphatase activity is of bacteriological origin, it was clearly preferable to avoid having to carry out this further test whenever reactivation had occurred, and particularly in view of the small amount of milk available.

The use of an antibiotic was considered and penicillin finally chosen and used at a level of 1 unit/ml. The experimental work leading to this choice is discussed in a later section.

Using penicillin at the above level it was found that reactivation occured at heating temperatures as low as 84° C., but no evidence was obtained at this stage of the occurrence of reactivation at lower temperatures.

This finding of reactivation after heating to as low a temperature as 84° C. was of some interest in that it is believed to afford the explanation of slightly high phosphatase results obtained some years ago on unpreserved samples of flash pasteurized milk from a certain country creamery. At the time no explanation could be found for these high results, but re-examination of the data obtained at that time makes it almost certain that the slightly high results were due to reactivation.

Storage time and temperature

In the original experiments on 'Uperized' milks from Switzerland the storage time was at least 1 week and in some cases several weeks' storage was given prior to examination of the milks. The reason for this was primarily bacteriological, since it was felt necessary to assess the ability of the milks to withstand prolonged storage. In the subsequent work using the capillary technique, it was clearly necessary, as has already been stated, to reduce this time to a minimum. Tests covering this point were made and a 2-day period was selected. Typical figures are given in Table 5.

Table 5

Storage time at 30° C. (days)	Reactivation level (2 hr. A/M test)
1	0·2+
2	0·3
3	0·4−
4	0·4
5	0·45
6	0·5
7	0·55

A few experiments were made to determine the optimum storage temperature, and all showed that this was approximately 30° C. The possibility that reactivation might occur within a few hours at temperatures higher than 37° C. followed by slow inactivation of the phosphatase, was also examined and these results are given in Table 6.

Table 6

Storage temp. (° C.)	Storage times (hr.)						
	½	1	2½	4	6	24	48
Cold store	—	—	—	—	—	<0·1	<0·1
18	—	—	—	—	—	0·15	0·1+
22	—	—	—	—	—	0·25	0·25
26	—	—	—	—	0·15	0·3	0·4−
30	—	—	—	0·15	0·15	0·3	0·4
37	—	—	0·15	0·1+	0·15	0·3−	0·3−
44	—	Nil	Trace	Trace	<0·1	Trace	Nil
52	Nil	Nil	Trace	Nil	Nil	Trace	Nil

These results showed not only that the optimum temperature of storage was approximately 30° C., but further that although reactivation may have commenced somewhat earlier at 37° C. than at 30° C. there was no indication of any more rapid reactivation at either 44 or 52° C. At these two higher temperatures practically no reactivation took place.

At the commencement of this paper reference was made to the fact that the original cans of 'Uperized' milk were stored at various temperatures before examination. Each can was, in fact, stored at two different temperatures, and a study of these storage temperatures and times, together with the corresponding L.B.U. value, showed the relative values of the latter to be in agreement with those expected from our experiments using capillary tubes as reported above.

It is also of interest to record here the L.B.U. values obtained on a further batch of cans of 'Uperized' milk before and after storage for 1 and 3 weeks at various temperatures. These are given in Table 7.

Table 7

(Initial L.B.U. value of milk = 2·8, after laboratory pasteurization = 1·0)

Storage temp. (° C.)	Kay-Graham L.B.U. values			
	1 week		3 weeks	
	Direct	After lab. pastn.	Direct	After lab. pastn.
4	3·4	1·0	2·8	1·0
22	13·0	1·1	14·0	1·1
26	21·0	0·9	23·0	1·0
30	21·0	1·2	25·0	1·0
37	23·0	1·0	18·0	1·0
55	1·4	1·4	1·6	1·8

These figures suggest that some inactivation of phosphatase occurs at 37° C. after 3 weeks, and in our experience Seitz-filtered cultures of bacteria containing bacterial phosphatase show this same effect.

The use of penicillin to prevent bacterial spoilage during storage

Reference has already been made to the use of penicillin in studying the occurrence of reactivation in raw milk shocked to temperatures below 120° C. Before its use was adopted, preliminary experiments were made to determine whether its presence would affect the following characteristics of milk phosphatase: (*a*) its hydrolytic power towards buffer substrate, (*b*) its heat resistance, and (*c*) its reactivability.

(*a*) Varying amounts of penicillin were added to pasteurized milk containing a small quantity of raw milk and both the Kay-Graham and A/M tests carried out:

Penicillin concentration, units/ml.

	Nil	0·1	0·2	0·5	1	5
K–G test	3·8	3·7	3·7	3·6	3·5	1·3
A/M test	0·3–	0·25	0·25	0·25	0·25	Trace

These figures show that no significant effect on the hydrolysis occurs up to a level of at least 1 unit/ml. as far as the present work is concerned, and are quoted because it has been claimed by Stoltz & Hankinson (4) that amounts of penicillin in raw milk of the above order can be detected by their effect on the phosphatase test.

(*b*) By heating raw milk with and without penicillin to a temperature of 62·8° C. for 15 min., it was found that the amount of residual phosphatase was unaffected by the presence of penicillin at a level of 1 unit/ml.

(*c*) A number of comparisons were made by shocking raw milks with and without penicillin under identical conditions. No effect on the level of reactivation was observed using a concentration of 1 unit/ml.

By using penicillin at this level of 1 unit/ml. very little trouble was experienced from bacterial growth during the storage period.

Bacteriological examination of shocked milk

During the first stages of this work all experiments were covered bacteriologically by plating out on milk agar at 30° C. The following procedure was used:

Each capillary was swabbed with alcohol, broken at each end with sterile forceps and the contents transferred into a weighed and sterile Durham tube. A rubber teat was then placed on the upper end of the capillary and the contents mixed before transferring

a small drop (0·01 ml. approximately) into a sterile Petri dish. The remainder of the milk was then weighed and the micro A/M phosphatase test applied.

The presence of phosphatase-producing organisms on plates can readily be detected by incorporating in the medium a small amount of disodium *p*-nitrophenyl phosphate (5), and this addition was usually made.

As the work progressed it became apparent that the milks were always sterile if the heating temperature was over 130° C., and accordingly plating was subsequently only carried out on milks heated to temperatures below this value.

Degree of reactivation

Throughout the earlier part of this work, milks shocked under apparently identical conditions and subsequently stored at 30° C. for the same length of time, showed a variable degree of reactivation. For instance, using a heating temperature of 135° C. and an immersion time of 20 sec., followed by 1 day's storage at 30° C. the values obtained varied from 0·1 to 0·6.

This variation was given a considerable amount of attention, for unless the cause was eliminated or understood, it was clear that many experiments designed to throw light on the whole problem of reactivation would be much reduced in value.

The question of technique was first considered, but despite a careful examination of all the likely variables associated with technique, no light was thrown on this variation. Among the factors studied were capillary size and wall thickness, uniformity of Durham tubes, rate of cooling, and cleanliness of glassware. The variation was well outside the error of reading, but since it was repeatedly found that replicates set up from the same milk at the same time gave good agreement, it was reasonable to conclude that the variation was due to the milk and not to any error in technique.

Examination was then made of a number of milks drawn from different sources, including both bulk raw milk from rail tanks and single churns direct from farms. It was found that the level of reactivation given by these milks varied considerably, and moreover that these differences were maintained when the storage period was extended to 7 days, i.e. the lower levels were not due to any lag in the development of reactivation during the early stages of storage.

The main factors influencing the level of reactivation have since been ascertained, but an account of this work will appear in a later paper.

The effect of Laboratory pasteurization on shocked milk

Laboratory pasteurization was usually carried out by immersion of the capillaries in a thermostatically controlled 62·8° C. water-bath for 35 min. In general, this method was applied when storage followed pasteurization as under (1) below, but when no further storage was necessary pasteurization was always carried out on an aliquot of the contents of at least two capillaries and was conducted in a Durham tube stoppered with a rubber bung and completely immersed, the remainder of the contents being tested for phosphatase.

(1) *Before storage* (*i.e. immediately after shocking*)

In most cases no reactivation occurred after 2 days' storage at 30° C. In one or two cases, however, reactivation did occur after 2 days, and it was noted that these particular

milks gave an abnormally high level of reactivation following normal shock treatment and storage. After 7 days at 30° C., however, some reactivation usually occurred.

Reference has already been made to the fact that with the 'Uperized' milks from Switzerland, different levels of reactivation were found when laboratory pasteurization was carried out after the high-temperature treatment, but prior to storage.

(2) *After storage* (*reactivated milk*)

Laboratory pasteurization was found to inactivate irreversibly the reactivated phosphatase. This was done on many occasions, and in every case no phosphatase could be detected immediately after pasteurization and no positive reaction developed on subsequent storage.

It can therefore be concluded that the results obtained with the commercial samples have been repeated and confirmed with the capillary tube technique.

III. THE REACTIVATION OF A BACTERIAL PHOSPHATASE

Having confirmed our findings on commercial samples of 'Uperized' milk by use of the capillary tube technique in the laboratory, it was thought of interest to ascertain whether bacterial phosphatases could also be reactivated by this method.

Organism

The organism used in these experiments belonged to the *Pseudomonas* group and was isolated from milk a few years ago. It is a very strong phosphatase producer, e.g. when grown in milk for 24 hr. at 22° C. it gives an L.B.U. value with the Kay-Graham test of 12–15 approximately, with a normal control value of $<1{\cdot}5$. Moreover, although the organism—designated 236—is non-heat-resistant, its phosphatase is very heat-resistant, a temperature of little less than 77° C. being necessary to bring about its inactivation in 30 min. in milk. Its optimum pH is 9 approximately in the presence of disodium *p*-nitrophenyl phosphate.

Experimental

Tests were made on: (1) nutrient broth cultures; (2) a mixture of equal volumes of nutrient broth cultures and sterilized milk; (3) sterilized milk cultures.

Since with milk phosphatase the average reactivation level was of the order of 0·2–0·4, i.e. equivalent to 0·5 % approximately raw milk, it was felt essential in these experiments to ensure, if possible, that sufficient bacterial phosphatase was present before shock treatment to enable reactivation of this order to be detected. Accordingly, measurements were made of the phosphatase content of nutrient broth and sterilized milk cultures of 236 after varying periods of incubation. It was found that the maximum amount of phosphatase produced by this organism in broth or sterilized milk culture was equivalent to 20 % raw milk, obtained after 10–14 days' growth at 22° C. Cultures of this age were therefore used in these experiments.

Since 236 is destroyed by laboratory pasteurization, and because reactivation of milk phosphatase had been shown to occur at temperatures as low as 85° C., the temperatures of shocking used first were from 85 to 120° C. No reactivation could be detected even after 7 days' storage at 30° C. in (1), (2) and (3). However, as this particular phosphatase is much more heat-resistant than milk phosphatase, it was thought that reactivation might occur after shocking to higher temperatures. Accordingly, higher temperatures were then used, namely 120–180° C., but though again no reactivation occurred using

nutrient broth cultures alone (1), reactivation did take place in the presence of sterilized milk (2) and (3).

Further experiments were made under (2), and it was found that whilst reactivation occurred in the presence of sterilized milk using temperatures of shocking in excess of 120° C., little or no reactivation occurred in the presence of pasteurized milk. Some reactivation took place in the presence of raw milk, but this was due to milk phosphatase as shown by its inactivation on laboratory pasteurization.

Reference will be made to additional experiments on this phosphatase and their possible significance in a later paper, but it must be stated here that the reactivation developed in the presence of sterilized milk was due to a phosphatase identical with that present in unheated cultures of 236 as regards its heat stability and pH optimum. At no time has any reactivation occurred after shock treatment of sterilized milk itself.

DISCUSSION

The time/temperature combinations required to inactivate the alkaline phosphatase present in raw milk are well-established facts, inactivation being virtually instantaneous at temperatures over 80° C. It is shown in this paper that when raw milk is rapidly heated to temperatures exceeding 85° C. a positive phosphatase reaction develops when the milk is stored at temperatures varying from 18 to 37° C. with an optimum at 30° C. approximately. Further, that this positive reaction occurs at all temperatures of heating from 85 to 170–180° C., but that this positive reaction is dependent upon the time of holding at these temperatures. It is also shown that the phosphatase responsible for this positive reaction is apparently identical with the alkaline phosphatase of raw milk. Attempts to obtain a positive phosphatase reaction following on shock heat treatment of a bacterial phosphatase have been successful in the presence of sterilized milk and again the phosphatase developed during storage is apparently identical with the unheated bacterial phosphatase.

This development of a positive phosphatase reaction during storage following the rapid heating to high temperatures has been termed reactivation, for there is no evidence of any difference between this developed phosphatase and its original counterpart.

The reactivation of enzymes is by no means a new phenomenon, for many cases are recorded in the literature (6, 7, 8), but it is believed that this is the first record of the occurrence of reactivation of alkaline milk phosphatase due to heat treatment.

Work on this reactivation which throws some light upon its mechanism is proceeding, and this will be published in a further paper.

SUMMARY

1. Commercial samples of milk of Swiss origin, sterilized by rapid heating to high temperatures, developed a positive phosphatase reaction on storage.

2. The optimum temperatures of storage for the development of this positive phosphatase reaction was 30° C.

3. These commercial samples were sterile when received and remained sterile during storage.

4. The developed phosphatase is apparently identical with the normal alkaline phosphatase of raw milk as judged by (*a*) heat stability, (*b*) pH optimum and rate of

hydrolysis at different pHs, (*c*) comparison of results by Kay-Graham and Aschaffenburg & Mullen tests.

5. This development of a positive phosphatase reaction in such milk has been termed reactivation and has been reproduced in the laboratory by a simple method and measured by a micro phosphatase technique based on the Aschaffenburg & Mullen phosphatase test.

6. Reactivation has been shown to occur over a wide range of heating temperature. No evidence of this phenomenon was obtained in either commercial samples of pasteurized milk (H.T.S.T. 161° F./15 sec. or Holder 145° F./30 min.) or laboratory pasteurized milks (145° F./30 min.).

7. Reactivation of a phosphatase of bacterial origin has been shown to occur.

8. A study of some of the factors affecting reactivation has been made and will be published in a further paper.

In conclusion the authors wish to express their thanks to Dr R. J. MacWalter for his constant and stimulating interest in this problem and in particular for his suggestion which led to the use of capillaries.

Finally, they wish also to express their thanks to Mr K. Gressly, General Manager of Oursina, Ltd., Berne, Switzerland, for the supply of samples of 'Uperized' milk.

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511. OBSERVATIONS ON THE SEROLOGICAL TYPING OF GROUP N ('LACTIC') STREPTOCOCCI

By C. A. E. BRIGGS and L. G. M. NEWLAND

National Institute for Research in Dairying, University of Reading

Serological methods have proved invaluable in the progressive definition and characterization of the group (N) of lactic streptococci by Mattick & Shattock (1), Shattock & Mattick (2), Swartling (3), Briggs (4) and Briggs & Newland (5). In recent years the importance of this group of organisms, not only in the field of dairying, has been enhanced by the work of Mattick, Hirsch, Berridge and their colleagues on the antibiotic nisin (6, 7, 8, 9, 10, 11, 12).

A rapid and reliable means of identifying and differentiating individual strains and types of group N streptococci would be of value. Although Hirsch & Grinsted (13) have classified certain 'inhibitory' lactic streptococci with particular reference to their antibiotic production, an overall taxonomic survey of the organisms comprising group N is still lacking. It was originally intended to undertake such an all-embracing investigation by serological typing. Our preliminary observations, however, showed a marked antigenic complexity and overlap among organisms of the group concerned, such that the somewhat ambitious study previously envisaged could not now be considered worth while.

We have confined our attention, therefore, mainly to those organisms of most general interest and practical importance. The observations recorded in this paper concern primarily the serological association between the classic strains of *Streptococcus lactis* and *Str. cremoris*, OJ and HP, and between strains in a representative collection of heterologous group N streptococci of importance as starter cultures and as sources of nisin.

METHODS

Bacterial cultures

The strains used in this work included those in the N.I.R.D. collection of freeze-dried cultures (14), together with the fresh isolations and acquisitions referred to in an earlier paper (5).

Biochemical and physiological tests

The biochemical and physiological tests employed have been mentioned and their value and significance discussed previously (5). We have continued to rely upon maltose fermentation, growth at 40° C. and the hydrolysis of arginine (15) for the physiological differentiation of *Str. lactis* and *Str. cremoris*.

Serological methods

Group sera

Group N precipitating sera for *Str. lactis* were prepared in rabbits using immunizing suspensions heated at 56° C. for 1 hr. (5). The 'absorption' or antigen-blocking technique of Briggs & Newland (5) was used in the preparation of immunizing suspensions for the production of group sera for *Str. cremoris*. These methods, and the procedures adopted for testing the sera for group antibodies and specificity have been described in detail already (5).

Type sera

Standardization of materials and technique is essential in work of the nature described here. It is difficult to standardize method and reagent, particularly extracts, and to determine level and degree of reaction in the precipitin test, whereas these are simple matters with the agglutination technique. Furthermore, a disadvantage of bacterial extracts is the uncertainty, in the first instance, concerning the quantitative and even qualitative representation therein of the different antigens of a given strain. For these reasons, with the added weight of anomalous results obtained with the precipitin technique during preliminary concurrent trials of the suitability for our purposes of precipitin and agglutination tests, we have discarded the former and resorted to agglutination alone.

Without regard to the variety of lactic streptococcus concerned, agglutinating type sera were prepared in every case against organisms heated at 56° C. for 1 hr. Immunizing suspensions were prepared by removing the cells from 24 hr. cultures in 1·0% dextrose Lemco broth (D.L.B.)(3) at 30° C. and resuspending them in 0·85% saline; the suspensions were standardized to an opacity equivalent to that of Brown's tube no. 10, and 1·0 ml. was injected intravenously into rabbits at 3- to 4-day intervals. Potent type sera were usually obtained after four inoculations. Frequent collection of blood and testing of sera were necessary during the course of inoculation in order to harvest sera at a high titre but before the appearance of group antibodies. Tests for the latter were made by the precipitin method with extracts of a representative selection of heterologous group N streptococci. Tests for potency were made by a simple tube-agglutination technique in dilutions of 1 : 40 to 1 : 5120, against standard formalinized broth suspensions of the homologous organism. As a rule, sera were harvested at a homologous titre of 1 : 5120 and, in a few instances, at 1 : 1280 as a minimum; they were preserved with merthiolate solution (final concentration 1 : 10,000) and stored at 4° C. Where necessary, sera were rendered type-specific by absorption with suspensions of cross-reacting strains.

Extracts

Hydrochloric acid extracts of the streptococci were prepared by Lancefield's method (16).

Agglutinating suspensions

Standard bacterial suspensions for tube-agglutination tests were prepared in bulk, formalinized (0·25%) and stored at 4° C. The organisms were grown in 0·2% D.L.B. at 22° C. for 18 hr. or longer, depending on the cell concentration then reached. The suspensions were standardized to contain 200,000,000 organisms/ml. (17) by optical density in a photoelectric absorptiometer calibrated for a variety of lactic streptococci.

Initial series of agglutination tests were made with equivalent formalinized broth (0·2% D.L.B.) and saline (0·85%) suspensions in parallel. Saline suspensions were considerably less stable on storage than those in broth, and gave less clear-cut results in the agglutination tests. Broth suspensions were therefore used throughout. A few strains failed to yield stable suspensions, even in broth. This difficulty was overcome by continued incubation of the culture in broth until twice the desired cell concentration, i.e. 400,000,000 organisms/ml., had been obtained, followed by filtration of the suspension through Whatman 54 filter-paper, this roughly halving the original concentration. In this connexion, also, satisfactory suspensions were prepared in a glucose phosphate broth of similar composition to that described by Colebrook (17), with the substitution of Difco beef extract for minced beef.

With one notoriously difficult strain (*Str. lactis* X13) the clumps of bacteria in auto-agglutinated suspensions were broken up and a homogeneous stable suspension made by the use of a magnetic shaker (18). 10 ml. of suspension was mixed with about 1 g. of fine glass beads and shaken for 1 hr. using a frequency of 50/sec. and a stroke *c.* $\frac{1}{2}$ in.

Absorbing suspensions

Absorbing suspensions were made by growing cultures at 30° C. for 48 hr. in 0·2% D.L.B. The broth cultures were standardized in the manner described previously to a concentration of 40,000,000,000 cells/ml. (17), formalinized and stored at 4° C.

Precipitin tests

Shattock's method (19) was used.

Agglutination tests

Following numerous trial-and-error tests the agglutination technique giving the most clearly read and consistently reproducible results was found to be 2 hr. water-bath incubation at 55° C. followed by standing at room temperature overnight. Eight dilutions of serum were employed, 1 : 40 to 1 : 5120, and saline controls. Where appropriate, screening tests were made at the first dilution only.

Agglutination absorption

All absorptions were made with serum dilutions of 1 : 20 and at the homologous minimum absorbing dose (M.A.D.) level of the serum concerned; M.A.D. were determined by Colebrook's method (17). Absorbing mixtures were incubated with repeated shaking for 1 hr. in a 37° C. water-bath and were then kept at 4° C. overnight before separation and titration of the absorbed sera.

RESULTS

Serological grouping

All the strains of streptococci examined were shown to belong to group N by precipitin tests with specific group sera for *Str. lactis* (2), *Str. cremoris* (5), and *Str. diacetilactis* (3).

Serological typing

The total collection of lactic streptococci numbered sixty-eight, and type sera were prepared for fourteen heterologous strains comprising varieties of *Str. lactis*, inhibitory and non-inhibitory, *Str. cremoris* and *Str. diacetilactis*. Because of the complexity of inter-reaction between even such small numbers of strains and sera as these it is not practicable to give here the details and progress of specific resolution of each. Instead, representative results, exemplifying the various observations, are given in the following sections.

Inhibitory Str. lactis *strains*

Type sera were prepared against five inhibitory strains of *Str. lactis*. Each reacted with the four heterologous strains, although in some cases the original unabsorbed sera possessed differing overall reactions. Table 1 shows, as examples, the reactions of the sera prepared against the nisin-producers, *Str. lactis* M3 and X13, and the strain of *Str. cremoris* 98/S1 with which M3 serum cross-reacted; also reciprocal absorptions of these sera, to type specificity in the case of M3. The presence of a common type antigen in the

inhibitory strains is apparent, while *Str. lactis* M3 clearly has an additional antigen not shared by X13. Since these absorptions were made at the homologous M.A.D. level of the sera concerned, the presence of an extra heterologous antigen in M3 is indicated rather than that M3 possesses the common antigen in greater proportion or concentration

Table 1. *Reciprocal absorption tests of* Str. lactis *and* Str. cremoris *type sera*

	Type sera								
	Inhibitory *Str. lactis* M3			Inhibitory *Str. lactis* X13			Non-inhibitory *Str. cremoris* 98/S1		
Agglutinating suspensions	Un-absorbed	Absorbed X13	Absorbed 98/S1	Un-absorbed	Absorbed M3	Absorbed 98/S1	Un-absorbed	Absorbed M3	Absorbed X13
Str. lactis M3	+	+	+	+	−	+	+	−	+
Str. lactis X13	+	−	+	+	−	+	−	−	−
Str. cremoris 98/S1	+	+	−	−	−	−	+	−	+

than X13. The fact that X13, unlike M3, did not react with 98/S1 also supports this view, and it appears that it was in fact a particular antigen in M3, not present in X13, which was responsible for its reaction with 98/S1. So far as the results in Table 1 are concerned, the antigenic structure of the relevant strains may be conventionally expressed as follows:

	Str. lactis M3	*Str. lactis* X13	*Str. cremoris* 98/S1
Antigen common to inhibitory strains	A	A	—
'Heterologous additional' antigen	B	—	B
(Group antigen	N	N	N)

Similar results were obtained with the other three sera for inhibitory strains of *Str. lactis*, and tests of the five sera with suspensions of the remaining inhibitory strains in the collection showed in fourteen (78%) out of eighteen cases the presence of the common type antigen apparently correlated with the property of nisin production. The exceptions will be referred to in the discussion. About forty non-inhibitory strains, whether *Str. lactis* or other variety, were tested against the five sera and none possessed the 'nisin' antigen, and none of the type sera prepared against inhibitory strains of *Str. lactis* reacted with *Str. cremoris* strains other than 98/S1.

Str. diacetilactis *strains*

Results similar in principle to those with the inhibitory *Str. lactis* strains were obtained with four *Str. diacetilactis* strains and type sera with regard to the presence of a common type antigen, and of a heterologous type antigen or antigens (in strain 54). The results of reciprocal absorption tests between two *Str. diacetilactis* type sera are shown in Table 2.

Table 2. *Reciprocal absorption tests of* Str. diacetilactis *type sera*

	Type sera			
	Str. diacetilactis 54		*Str. diacetilactis* no. 2	
Agglutinating suspensions	Unabsorbed	Absorbed no. 2	Unabsorbed	Absorbed 54
Str. diacetilactis 54	+	+	+	−
Str. diacetilactis no. 2	+	−	+	−

Confirmatory results were obtained with the remaining diacetilactis type sera, and with tests using the four type sera and suspensions of other strains in the collection.

Str. cremoris *strains*

The results of the reciprocal absorption tests given as examples in Table 3 show the sharing of a common antigen between the strains of *Str. cremoris* H_{22} and J, and the presence of an additional unshared antigen in strain H_{22}. *Str. cremoris* H_{22} failed to give

Table 3. *Reciprocal absorption tests of* Str. cremoris *and* Str. lactis *type sera*

	Type sera								
	Str. cremoris H_{22}			*Str. cremoris* J			Inhibitory *Str. lactis* M3		
Agglutinating suspensions	Un-absorbed	Absorbed J	Absorbed M3	Un-absorbed	Absorbed H_{22}	Absorbed M3	Un-absorbed	Absorbed H_{22}	Absorbed J
Str. cremoris H_{22}	+	+	+	+	−	+	+	−	+
Str. cremoris J	+	−	+	+	−	+	−	−	−
Str. lactis M3	+	+	−	−	−	−	+	−	+

antigen-antibody reactions with inhibitory strains of lactic streptococci other than M3; thus the heterologous antigen in H_{22} cannot be confused with the common antigen of the nisin-producing strains. Results confirming the structural principle of one common antigen and another heterologous antigen(s) were obtained in tests of further *Str. cremoris* type sera with the varieties of lactic streptococci in the test collection.

Str. lactis *OJ and* Str. cremoris *HP*

The serological association between the classic strains of *Str. lactis* and *Str. cremoris*, OJ and HP, proved of great interest in that it demonstrated differing proportional representation of common antigens in contradistinction to the presence of simple proportionally equivalent common or heterologous antigenic components in the lactic streptococci. Reciprocal absorption tests of OJ and HP type sera at one, two, three and

Table 4. *Reciprocal absorption tests of* Str. lactis *OJ and* Str. cremoris *HP type sera at* 1, 2, 3 *and* 4 *times the homologous* M.A.D.

	Type sera									
	Str. lactis OJ					*Str. cremoris* HP				
	Absorbed with HP at four levels					Absorbed with OJ at four levels				
Agglutinating suspensions	Un-absorbed	Homologous M.A.D.*	2 × M.A.D.	3 × M.A.D.	4 × M.A.D.	Un-absorbed	Homologous M.A.D.*	2 × M.A.D.	3 × M.A.D.	4 × M.A.D.
Str. lactis OJ	+	+	+	+	−	+	−	−	−	−
Str. cremoris HP	+	−	−	−	−	+	+	+	+	−

* Homologous M.A.D. in both cases = 10^9 cells/ml. 1:20 dilution of serum.

four times the homologous M.A.D. showed these strains to be mirror images of each other so far as their type antigens are concerned (Table 4). Their antigenic structures may be conventionally expressed as:

Str. lactis OJ		*Str. cremoris* HP
4C, 1D	Type antigens	1C, 4D
(N	Group antigen	N)

These observations are of particular moment since *Str. lactis* OJ and *Str. cremoris* HP have for so long been regarded as grossly different varietal 'type' strains of group N streptococci. According to the plan above, antigen 'D' evidently represents the antigen of *Str. cremoris* which, in work on the serological grouping of that organism according to Lancefield's principles, so consistently gave cross-reactions with the streptococci of groups K and L (see (5)).

OJ and HP failed to give serological type reactions with any other strains of group N streptococci, save for evidence of the common antigen between HP and other strains of *Str. cremoris*. In this connexion it should be mentioned that *Str. lactis* OJ was not an inhibitory strain, although this does not explain its failure to react with strains of *Str. cremoris* with which HP shared a common antigen; perhaps proportional differences in antigenic composition are concerned in this apparent anomaly, in that OJ possesses only one 'unit' of the 'cremoris group' antigen 'D' compared with the four units of *Str. cremoris* HP. The conventional expressions of antigenic structure above may or may not be, along with the group antigen indicated in parentheses, complete, and include all antigens that the respective strains possess. In any event, *Str. lactis* OJ and *Str. cremoris* HP appear so far to be among the least complicated, antigenically, of the group N streptococci. The question whether *Str. lactis* OJ possessing to a lesser degree (i.e. compared with strains of *Str. cremoris*) the 'cremoris group' antigen, should be regarded as a variety of that species will be referred to later.

DISCUSSION

Although, for reasons already stated, this work is far from complete, the basic principles of the type antigen design in the lactic streptococci seem reasonably clear. In general, all strains of a particular named variety share a common antigen. So far as can be ascertained at this point there is, of course, no overlap between the common antigens representing or defining particular groups. There is, however, considerable complexity in that individual (clearly not all) strains of a particular group may possess heterologous antigens, distinct from the 'group' antigen, and these may be present, again as heterologous antigens, in individual strains of other groups. Sharpe (20) has described the occurrence of a common type antigen in certain streptococci of groups D and N; strains of the latter group were serologically more complex than those of group D, possessing a similar but more complex type antigen, or, alternatively, a presumably equivalent common antigen together with an additional heterologous type antigen. Sharpe suggested that 'other group N streptococci may have the same intricate antigenic pattern'. Our observations closely resemble Sharpe's in plan and principle, although they relate to strains of individual species within group N, and not to inter-relationships between members of separate (Lancefield) groups. Unequal quantitative representation of common antigens has also been found in the lactic streptococci; this has been demonstrated in the serological association between only two strains, but may apply much more widely than our limited observations indicate. The ultimate possible combinations and varieties of antigenic relationship between individuals and groups of strains seem infinite and resemble, if not surpass, those found in the *Brucella* group of organisms. As previously mentioned, it is impossible in a communication of this nature to list the multiplicity of reactions obtained with such a complex selection of organisms and, since the examples quoted were chosen for their clarity, one should beware of a false impression

of simplicity. Perhaps it is not out of place here to express the opinion that, beyond a certain point, an investigation of this nature becomes not only uneconomic, as already suggested, but of limited practical importance, and chiefly therefore of academic interest.

So far as the present work is concerned, it is important that type sera, for rapid identification and 'labelling' purposes, can be prepared for a variety of commercially important strains of lactic streptococci, particularly inhibitory strains such as *Str. lactis* M3 and X13, and, for example, common starter components. Two cultures of *Str. lactis* X13 were used in this investigation; one was given to us by Dr A. Hirsch, then working on the inhibitory streptococci at this Institute, and the other was kindly sent to us by Monsieur G. Mocquot of the Station Centrale de Technologie Agricole, Paris. It was of interest, although they were originally the same stock, that these cultures gave identical serological reactions. As stated on p. 192, four out of eighteen nisin-producing strains of *Str. lactis* were not shown to contain the antigen common to the inhibitory strains in general. These were weak nisin producers, and, if the common 'inhibitory' antigen be concerned, as one may suppose, in the physiology of the bacterial cell with regard to nisin production, although nisin itself does not appear to be antigenic, this suggests a poor development of that antigen and may explain our inability to detect its presence in the strains in question.

As Hirsch & Grinsted (13) have pointed out, it is interesting to contemplate the possible role, in the serological reactions described, of the M substance, which Lancefield (21) has shown to be responsible for the type-specificity of certain group A streptococci, and whose properties resemble those of nisin in that both are proteins, soluble and heat-stable in acids (13). With regard to differing proportional representation of common antigenic components in lactic streptococci (although this has not yet been demonstrated in nisin-producing strains) it may be significant that Berridge (6) has already shown that crystalline nisin has at least two components, which may occur in different proportions in different strains.

It is worthy of note that, although the approach and ultimate object were different in each case, our specific results, particularly with individual strains, agreed convincingly with those of Hirsch & Grinsted (13) with certain inhibitory group N streptococci; and with those of Swartling (3) with the citric acid fermenting varieties which he recommends should be given species rank under the name *Str. diacetilactis*.

Str. lactis OJ and *Str. cremoris* HP were shown to possess the same type antigens but in inverse proportion. This may well account for the narrow and sometimes unstable dividing line between them, based on their physiological and biochemical properties (3, 5, 22). Environment may influence the activity of different antigenic components to different degrees, such that the physiological activity of a particular antigen substance, although present in only small amount like the 'cremoris group' antigen in *Str. lactis* OJ, may overshadow that of an antigen present in greater abundance. Associated cultural and antigenic variations have been found in haemolytic streptococci by Dawson, Hobby & Olmstead (23).

For these reasons, as well as that of priority, the specific epithet *Str. lactis* must remain. Little is known still of the influence of medium and environment in general on *Str. lactis* and *Str. cremoris*; an intensive study of these factors would be valuable if only in confirming that these organisms are distinct and relatively stable individuals. Hirsch (24) records the isolation of *Str. cremoris* from milk produced in the Jura mountains of France,

showing the occurrence of this organism in the 'wild' state, and in another communication (25) shows that *Str. cremoris* is particularly sensitive to the antibiotic nisin produced by *Str. lactis*, and that there is strong mutual inhibition between these two organisms. These observations, together with the evidence presented in this paper, suggest that the possibility of *Str. lactis* and *Str. cremoris* being mere mutants of a single original variety can no longer be considered.

Although the work described here has, in the main, served only to demonstrate considerable antigenic complication in the lactic streptococci, certain observations are of distinct importance. So long as serological methods remain a tool and not objects in themselves, and so long as that which is of practical application can be distinguished from that which is not, such techniques will continue to be of value and yield results of significance in appropriate circumstances.

SUMMARY

1. Agglutinating type sera were prepared in rabbits for fourteen representative strains in a collection of sixty-eight group N ('lactic') streptococci.

2. Common type antigens, distinct for each named species, were found in strains of *Str. lactis* (inhibitory varieties), *Str. diacetilactis* and *Str. cremoris*.

3. Certain individuals in each of the named 'groups' contained additional heterologous antigens, some of which were identical to heterologous (but not the common) antigens in strains of other groups.

4. Quantitative differences in the representation of common antigens were also found.

5. *Str. lactis* OJ and *Str. cremoris* HP were shown to be mirror images of each other so far as their type antigens are concerned, containing two common type antigens in inverse proportions of 4 : 1 respectively. *Str. lactis* OJ possesses the 'cremoris group' type antigen, and the implications of this observation are discussed.

6. The existence of various common and heterologous antigens, the latter showing considerable overlap among varietal groups, and of unequal quantitative representation of shared antigens show the lactic streptococci to be serologically very complex organisms. Despite such complication, observations of practical importance emerge.

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547·915

512. A PHOTOELECTRIC METHOD FOR DETERMINATION OF FAT ALDEHYDE VALUES

By R. M. DOLBY

The Dairy Research Institute (N.Z.), Palmerston North, New Zealand

(With 3 Figures)

When the Schibsted fat aldehyde test (1) is applied to butter-fat samples, accurate matching of the developed colour against the colour standard is prevented by the yellow colour of the butterfat. The difference in tint between sample and standard is specially pronounced with samples of low aldehyde value.

Mummery (2) has attempted to improve matching by the adoption of a standard of a more yellowish colour. For this purpose he used a buffer of pH 7·8 instead of pH 8·3 in which to dissolve the cresol red for the colour standard. This change makes it easier to obtain colorimeter readings on samples of medium aldehyde value but gives little improvement with samples of low aldehyde value. It seemed likely that a photoelectric method might prove more satisfactory.

Absorption values were determined with a Beckman spectrophotometer on samples of butterfat dissolved in petrol ether, as for the fat aldehyde test, before and after the addition of the rosaniline reagent. The curves obtained for a sample having a high aldehyde value are given in Fig. 1. The absorption curve for butterfat alone (lower curve) has a peak at 450 mμ corresponding to the absorption maximum for carotene. The absorption of the sample after addition of rosaniline reagent (upper continuous curve) gives the combined absorption due to butterfat and to aldehyde colour. The curve for absorption by aldehyde colour alone (broken line) has a peak at 510 mμ. At this wave-length the absorption by butterfat is almost negligible.

The curves for a butterfat sample of rather lower aldehyde value are given in Fig. 2. The peak values correspond with those of the first sample. It is evident that measurements made at 510 mμ will be very little affected by the colour of the butterfat. A correction may be made by subtracting from the density reading the density value of the butterfat solution at the same wave-length.

The absorption curves for Schibsted's colour standard (cresol red in buffer at pH 8·3) and for Mummery's modified colour standard (pH 7·8) are given in Fig. 3. The cresol red curves show peaks at 440 and at 570 mμ (corresponding respectively to the acid and alkaline forms of the indicator). Mummery's colour standard with an increased yellow component has a greatly reduced red component. It would therefore lead to a considerably higher aldehyde value for a given sample of fat than Schibsted's colour standard.

It is not possible to use Schibsted's standard as a standard in the photoelectric determination of aldehyde values since the absorption maximum for cresol red is quite different from that of the rosaniline-aldehyde reagent. The density value for a 0·1% fat solution in a 1 cm. cell could be used instead of the aldehyde value. If, however, it is desired to express the results in a manner which permits comparison with those obtained by the original Schibsted method, the following procedure may be adopted:

A sample of butterfat of high aldehyde value is selected and its aldehyde value

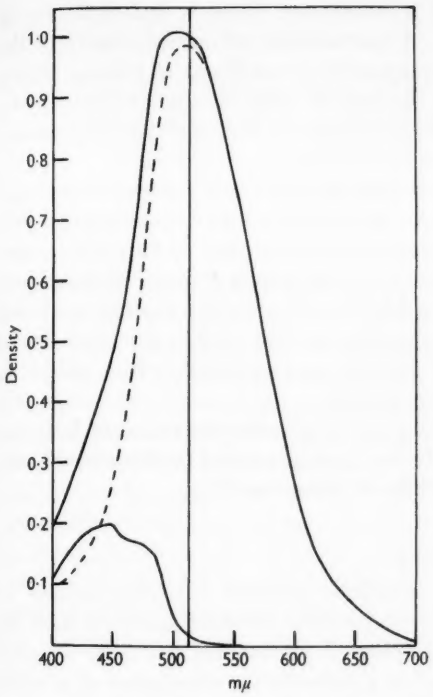

Fig. 1

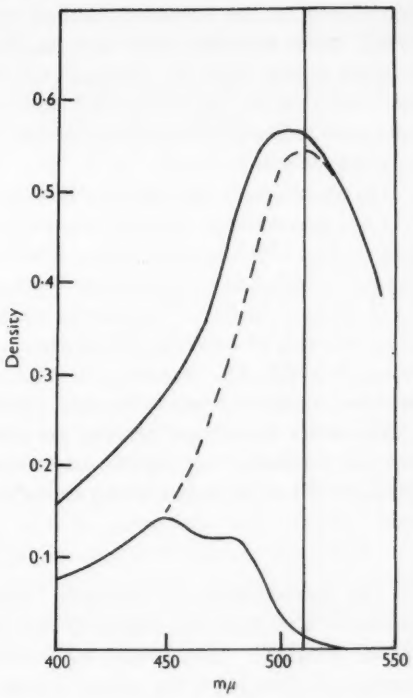

Fig. 2

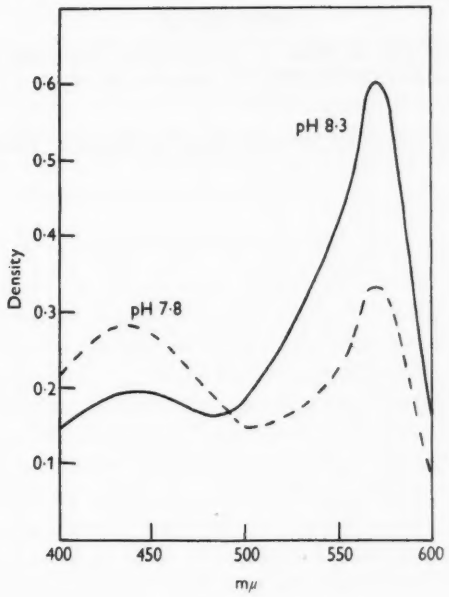

Fig. 3

determined by the Schibsted method using a colorimeter. There is little difficulty in visual colour matching with such samples. A measurement of optical density of the solution is then made in a photoelectric instrument at a wave-length of 510 mμ. Using the two values for this sample as a standard, the density values of other samples may be converted to Schibsted fat aldehyde values on the assumption that density is proportional to aldehyde value.

The photoelectric method may be employed with any instrument, provided the band width is not excessive. Satisfactory results have been obtained with a Spekker Absorptiometer using Ilford Spectrum Filter 604. A correction for absorption by butterfat is more necessary with a filter photometer than with a spectrophotometer, owing to the greater band width of the filter. The correction may be determined by taking a reading on a petrol ether solution of butterfat of the same concentration as that used in determination of aldehyde value. The correction is small and does not vary appreciably from sample to sample. A constant correction may, therefore, be used.

The method described permits the measurement of fat aldehyde values of butterfat samples which are very slightly oxidized and which, by the original method, would have given erratic or nil values owing to the difficulty of colour matching.

SUMMARY

In the determination of aldehyde values of slightly oxidized butterfat samples by Schibsted's method, the colour of the butterfat prevents accurate matching with the colour standard. Interference by butterfat colour is eliminated, and more accurate results are obtained if the colour is measured in a photoelectric colorimeter at a wave-length of 510 mμ. A small constant correction for the absorption by butterfat at this wave-length may be made.

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513. THE EFFECT OF DIFFERENT CREAM TREATMENTS DURING THE PASTEURIZATION PROCESS ON THE SIZE DISTRIBUTION OF FAT GLOBULES IN CREAM AND BUTTER

By R. M. DOLBY

The Dairy Research Institute (N.Z.), Palmerston North, New Zealand

(With 2 Plates)

The treatment applied to cream for buttermaking prior to churning usually causes some change in the size distribution of the fat globules. As a result, it may alter the churning characteristics of the cream and the proportion of fat lost in the buttermilk. The fat losses in the churning of cream which has been pasteurized by direct steam in a Vacreator* are usually higher than those in the churning of flash-pasteurized cream, even where measures have been taken to avoid any extra dilution during Vacreator treatment.

Sargent (9) showed that the fat globules in Vacreator-treated cream were of smaller average diameter than the globules in flash-pasteurized cream from the same bulk supply. The proportions of large and small globules in the two types of cream, however, were not reported.

The effects of some other types of pasteurizers and preheaters, and of pumping, on the fat globules in cream were investigated by Rahn (7) and by Lyons (6). It was shown that the agitation of hot cream taking place in a beater-type flash pasteurizer had little effect on the proportion of small globules but, by causing clumping, greatly increased the proportion of fat present as large globules (over 8μ in diameter).

Preliminary investigations showed that determination of size distribution of fat globules in creams containing large globules or aggregates presented special difficulties that were not present with milk or freshly separated creams. A study of methods of preparing the sample therefore became necessary.

Methods of determining size distribution of fat globules

The earliest measurements of fat globules in milk and cream were restricted to the estimation of the average globule diameter from a count of the number of globules in a known volume of the diluted sample. Direct measurement of globule diameters and calculation of the proportion of globules in each size class was made by Van Dam & Sirks (13) who placed the diluted sample in a counting chamber 0·2 mm. deep and allowed the preparation to stand for a sufficient time for the fat globules to rise to the under surface of the cover-glass before they made their observations. Rahn (8) modified this method by using an ordinary microscope slide and cover-slip so that the diluted sample was present in a thin film. He did not attempt to bring the globules to the upper surface of the preparation before observation. Schneck & Kohlhardt (11) also used a slide and cover-slip, but allowed the globules to rise so that they might be more readily photo-

* 'Vacreator' is the trade-mark for the Murray 'Vacuum Pasteurizer'.

graphed. Later workers have used modifications of the methods of either Van Dam & Sirks or of Rahn:

(*a*) A deep preparation in a counting chamber or haemocytometer, usually 0·1 mm. deep. The counting chamber was held at 30–40° C. for 1–2 hr. before observation, to allow rise of fat globules, e.g. Lyons (6).

(*b*) A thin preparation, 0·01–0·03 mm. deep, on a microscope slide. In this preparation the globules were distributed at all depths during observation, e.g. Schmidt (10).

Schmidt considered that the creaming procedure was a waste of time as the high viscosity, even at 40° C., of the gelatin solution used as diluent would prevent the rise of the smallest globules in the period available. To avoid omission of these small globules it was, in his opinion, still necessary to search the whole depth of the preparation. Schmidt concluded that, for milk, a film thickness of 20–25 μ was suitable, since it was easily possible to focus from top to bottom of such a preparation.

The creaming operation, as well as involving delay, may allow changes to take place in the fat globules. Lyons (6) has admitted that closely attached globules (clumped by agitation of cream) may coalesce during the period in which the slides are warmed to assist rise of globules.

Diluent. Milk samples must be diluted to some extent and cream samples to a greater extent, to space the fat globules sufficiently for counting or measurement. The diluent must have a sufficiently high viscosity to prevent Brownian movement of the globules while under observation. Glycerol has been used by a few workers but the majority have chosen gelatin solutions. This preference is probably due to the fact that fat globules show sharper contrast in gelatin than in glycerol solutions, owing to the greater difference in refractive index between fat and medium.

The extent of dilution of the sample and the concentration of gelatin required vary according to the depth of preparation to be used. Where all the globules from a 0·1 mm. depth are to be allowed to rise into one plane, a much higher dilution and a lower concentration of gelatin are necessary than where the globules are to remain distributed through the depth of the preparation. Lyons diluted milk 100 times with $1\frac{1}{2}\%$ gelatin, while Schmidt made a total dilution of only 5 times using 3% gelatin. Dilutions for cream must be 8–10 times as great as those for milk.

Schmidt considered that the most regular distribution of fat globules would be obtained at the minimum dilution, basing this opinion on the observation of Kreveld (5) that repulsive forces between fat globules in milk make their arrangement more regular than in a random distribution.

The fact that gelatin promotes clustering of fat globules would appear to render inadvisable the use of too concentrated a cream preparation, since clustering is more likely to take place the more closely the fat globules are packed.

The thin-film method has the advantage that ordinary microscope slides can be used and that no special microscope equipment is then necessary. With haemocytometer cells the best definition at high magnification can only be obtained if the condenser of the microscope is capable of focusing through the thick glass of the cell, and the objective, unless oil-immersed, is corrected for the thick cover-glass usually necessary on such cells.

The thinness of film that can be safely used is limited by the size of the larger fat globules in the preparation. If the depth of the film is less than the diameter of the larger globules, these globules will be flattened and their apparent diameter increased. A cor-

rection can be applied to measurements on such globules but, as will be shown later, any flattening of globules seems to decrease their stability and so should be avoided.

Measurement of diameters of fat globules

For measurement of globule diameters an ocular micrometer scale has been widely used (Van Dam & Sirks (13), Rahn (8), Lyons (6)). Globules lying close to the scale were measured and a further number were then brought on to the scale by moving the stage. This method has the disadvantages that it involves considerable eyestrain, that the magnification cannot always be adjusted so that a scale division represents a whole number of microns, and that there is a risk of overlooking some globules and of measuring others twice.

Other workers (Turner & Haskell (12), Schneck & Kohlhardt (11)) have used a photographic method. This is convenient in that it permits measurement of globules on an enlargement of suitable magnification, but it can give misleading results unless all the globules in the preparation have been brought into one focal plane. As the depth of focus of a 4 mm. objective is only about 2 μ, even allowing for some reduction of aperture, any small globules above or below this plane will be out of focus or invisible in the photograph. Larger globules lying partly in this plane, although their centres are some distance from it, will, however, appear in the enlargement. The photographic method thus will show too small a proportion of small globules unless a creaming technique has been used which can be relied on to bring all the small globules to the top of the counting chamber.

Projection methods which allowed easy observation with both eyes and permitted focusing up and down to measure all globules in the depth of the preparation have been used by other workers. They projected the image on to a white card or a ground-glass screen on which a scale was ruled. Schmidt (10) followed the latter method but used transparent graph paper on a clear glass plate. The magnification was adjusted to $\times 1000$ so that globule diameters in microns could be read directly from millimetre graph paper. The observations were made in a darkened room and, according to Schmidt, an arc lamp was required to give sufficient illumination to show the smallest globules clearly.

An Abbé camera lucida drawing apparatus has been employed by Whittlestone (14, 15). Though allowing only monocular vision, this method is otherwise convenient. It requires very simple equipment and can be used in a normally lighted room with an ordinary microscope lamp. The distance between the drawing board and the microscope can be adjusted so that the apparent magnification is $\times 1000$. Sizes of globules can be read off from graph paper attached to the drawing board, or, alternatively, each globule can be recorded on paper by a line equal to its diameter. With the latter method a field containing 200–300 globules can be recorded in a few minutes. The omission of any globules can be readily detected. Measurement of globule diameters from the record sheet can be done later at any convenient time.

EXPERIMENTAL

Choice of method

A preliminary examination was made of the haemocytometer method, as used by Lyons (6), to determine whether, in this method, any of the smallest fat globules would escape observation. A sample of skim milk was diluted 10 times with 1½% gelatin solution and placed in a haemocytometer (depth 0·1 mm.). The haemocytometer was held in an incubator at 30° C. for 2 hr. to allow rise of fat globules, and was then examined under

a microscope, the condenser of which was fitted with a special top lens designed to give satisfactory illumination with counting chambers.

It was found that, while all the larger globules had risen to the under surface of the cover-slip, about one-third of the smallest globules (1 μ and under) remained distributed through the medium. It was not easy to detect all the small globules below the surface, and as their Brownian movement was not entirely eliminated by the concentration of gelatin present, their measurement was difficult. It seemed likely that this method would not show the full number of small globules in cream. This conclusion was confirmed in a later experiment in which a comparison was made of fat globule distributions in cream estimated by Lyons's method and by the microscope slide method described later in the present paper. The cream sample was a freshly separated raw cream. The results in Table 1 show that the haemocytometer method gave a somewhat lower percentage of globules in the lowest size class (0·5 μ) than did the microscope slide method. The agreement otherwise between the two methods was very close.

Table 1

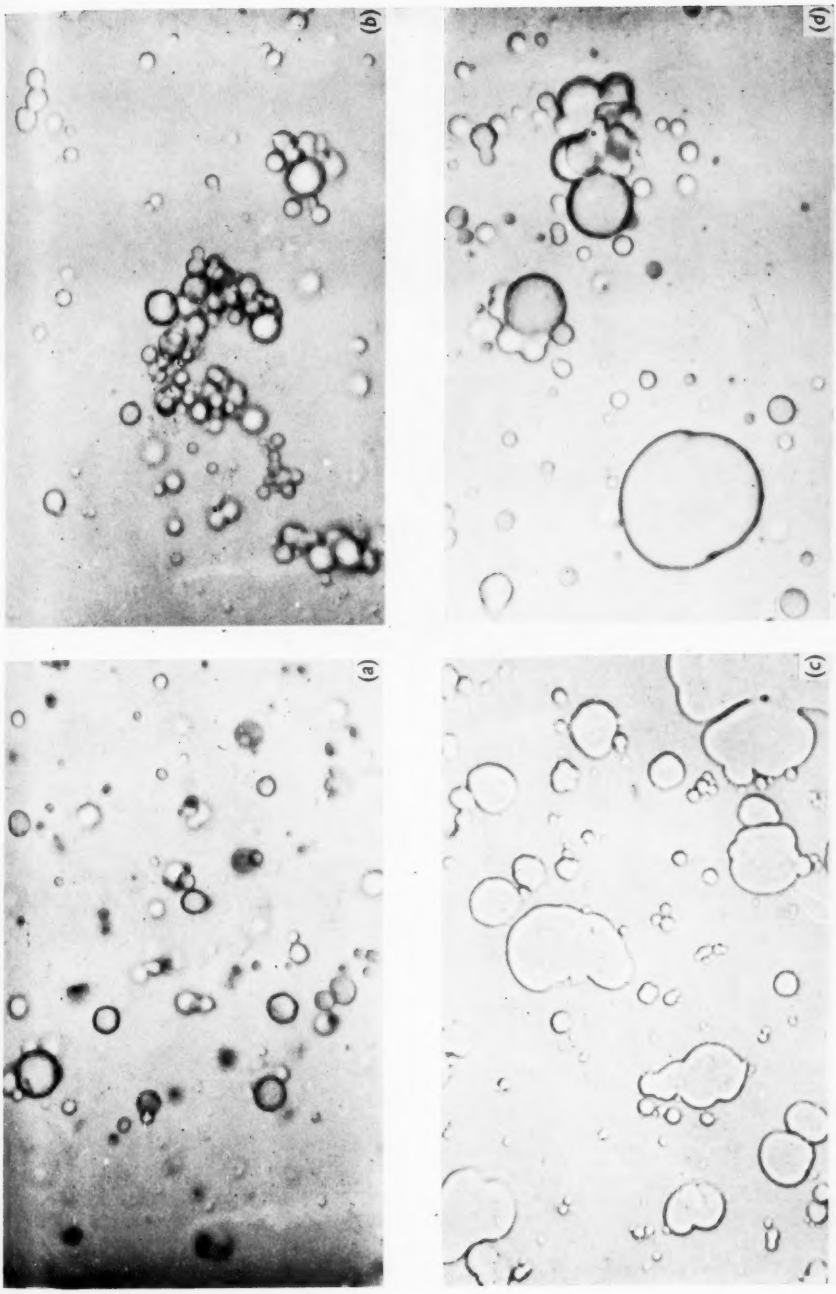
Globule diameter (μ)	Haemocytometer		Slide	
	No. of globules	Frequency (%)	No. of globules	Frequency (%)
0·5	123	14·0	169	18·9
1	78	8·9	63	7·0
1·5	81	9·2	91	10·2
2	169	19·2	186	20·8
3	207	23·5	186	20·8
4	124	14·1	129	14·4
5	58	6·6	43	4·8
6	30	3·4	21	2·3
7	6	0·7	5	0·6
8	2	0·2	2	0·3
9	1	0·1	—	—
Total	879		895	

As the correct estimation of the proportion of the smallest globules is particularly important in the examination of Vacreator-treated creams, it was decided to use only the microscope slide method in further work. This method is also more convenient, since a number of slides can be set up at the one time and, as pointed out earlier, there is less difficulty in microscopic examination.

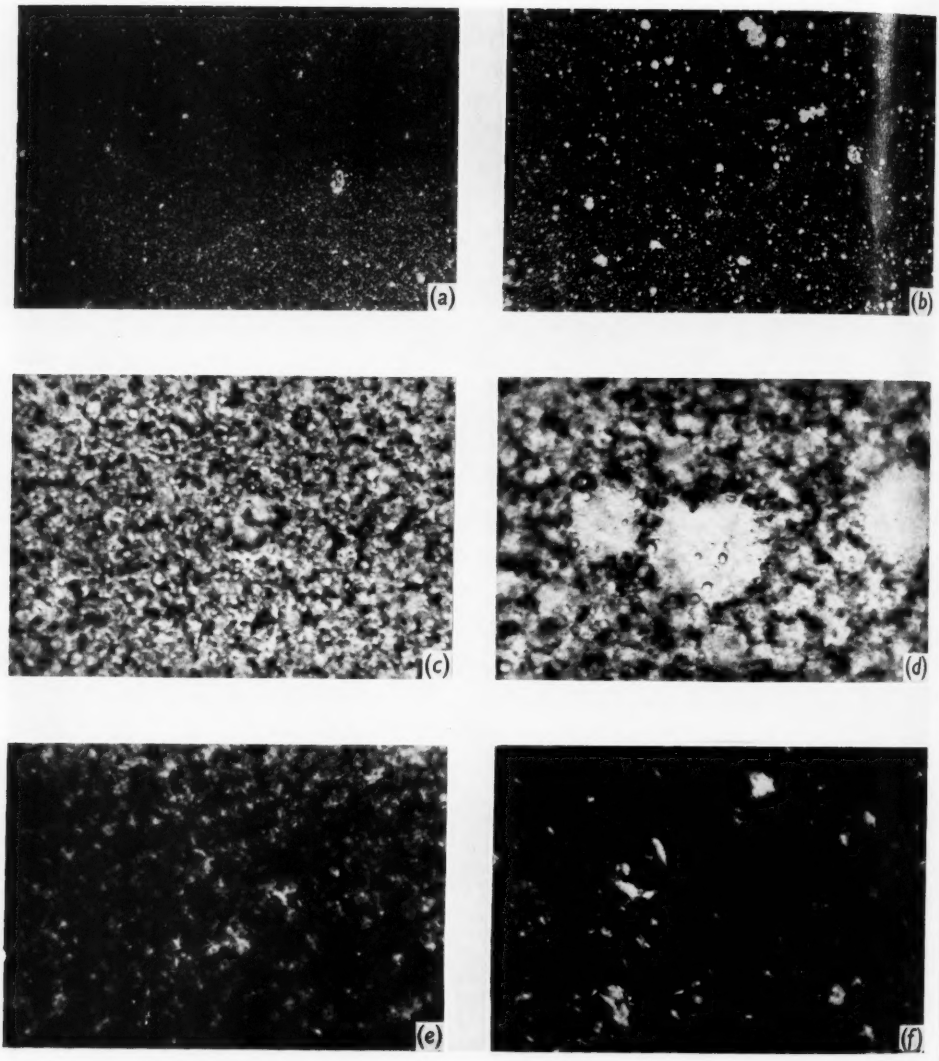
Preparation of samples

It was found that when a liquid gelatin dilution of cream was placed on a slide, flow continued for some time after the cover-glass was applied. Fat globules collected in clusters while this flow was taking place. Clusters formed in this way are shown in Pl. 1 (*b*). Globules in these clusters would be impossible to count or measure. If the gelatin mixture was cooled to the thickening stage before the sample was placed on the slide, there was no migration or clustering of fat globules after the cover-glass was applied (Pl. 1 (*a*)).

An even more serious objection to the preparation of slides from a liquid dilution was found with samples containing large fat globules or aggregates. With such samples when the cover-glass was applied the larger globules tended to remain stationary while the smaller ones flowed out with the liquid towards the edges of the cover-glass. This segregation of globules made it impossible to obtain representative fields.



(*a*) Well-dispersed globules in slide made from thickened gelatin dilution of cream. × 600. (*b*) Clusters of globules formed on slide set up from liquid gelatin dilution. × 600. (*c*) Coalesced globules formed on slide on which gelatin had remained liquid. (*d*) Large fat aggregates formed in flash-pasteurized cream. × 600.



(*a*) Vacreator butter. ×36. (*b*) Flash-pasteurizer butter. ×36. (*c*) Vacreator butter. ×450. (*d*) Flash-pasteurizer butter. ×450. (*e*) Vacreator butter by polarized light. ×450. (*f*) Flash-pasteurizer butter by polarized light. ×450.

If the gelatin had thickened or completely set before the sample was transferred to the slide, this segregation did not occur. Where a solid gelatin sample was taken it tended to be crushed by the cover-slip rather than to flow out, and the film showed cracks along which streamed liquid which had exuded from the gel. The most satisfactory results were obtained with gelatin mixtures which had not quite set and so could still flow.

When the gelatin mixture on a slide remained liquid for any length of time or was remelted by exposure to high atmospheric temperature, the fat globules tended to coalesce. This change occurred most readily in very thin preparations in which some of the largest globules were flattened between slide and cover-glass. Pl. 1 (*c*) shows the appearance of a diluted cream containing 1·8% of gelatin in a counting chamber 10 μ deep after being held for some hours at 65–70° F.

It was concluded from these observations that the concentration of gelatin should be sufficiently high to ensure that on cooling there would be a rapid formation of a gel which would remain solid at the highest atmospheric temperature to which the slide would be exposed. The solution should be cooled till thickened before transfer of the specimen to the slide. Gelatin concentrations of 4–6% were found suitable.

It was noticed that there was a possibility of some of the fat becoming churned during the cooling of the gelatin dilution of the cream, especially if the solution was stirred to prevent rise of fat and to facilitate heat transfer. The churning tendency was most pronounced in mixtures containing higher percentages of fat (e.g. 4%), but was not noticeable in more dilute mixtures (e.g. 0·5–1% fat).

Kreveld (4) has observed that in the gelatin used for dilution there could form, especially on long standing, fine globules which could not be distinguished from the smallest fat globules. To avoid formation of such globules he recommended the addition of a solution containing 2% sodium citrate and 0·1% sodium hydroxide to the gelatin solution. The precaution has been taken in the present investigation. Kreveld also advised heating the diluted samples to 65° C. to deaerate the mixture. This method has also been followed since it is desirable to eliminate air bubbles, and it was found that use of a vacuum for this purpose caused churning of some of the fat.

Creams containing large fat aggregates or partially churned fat are difficult to sample satisfactorily and microscopic fields of such samples cannot be expected to be representative. Some means of removing these fat aggregates before sampling for the microscopic preparation is therefore desirable. The method used in the present investigation was to allow creaming of the sample, after dilution with water, for a short time at a temperature at which clustering of fat globules would be low. The layer of cream or oiled-off fat was discarded, the proportion of fat removed being determined by fat estimation.

Details of method adopted

Samples were transferred to the laboratory without delay and were diluted within 15–20 min. from the time they were taken.

A 20 ml. portion of the cream was mixed with 180 ml. of water at 40° C. Part of the diluted cream was retained for fat estimation and 100 ml. of the remainder was placed in a separating funnel and allowed to stand. After 15 min., the lower 90 ml. was run off, a 2 ml. sample for microscopic examination removed and the remainder held for a further fat determination. The fat estimations were made by the Gerber method. The difference between the two results indicated the amount of fat which had risen while standing.

With samples which were not expected to contain large fat globules or aggregates the creaming operation and fat estimations were omitted.

Two ml. of a solution containing 4% sodium citrate and 0·2% sodium hydroxide and 6 ml. of a solution containing 10% gelatin and 1% phenol were placed in a test-tube and heated in a water-bath to 65° C. The 2 ml. sample of diluted cream was added and the contents of the tube were mixed, using a stirring rod with a bulb at its lower end. The tube was left in the bath for 5 min. and was then cooled in ice water with very slow stirring, care being taken not to incorporate any air. When the gelatin began to thicken, the stirrer was withdrawn and the tube was taken from the bath. A suitable quantity of the gelatin mixture was taken out with a platinum loop and placed on a microscope slide. A circular cover-glass was applied and pressed down gently. The quantity of sample and the pressure were adjusted so as to give a preparation approximately 20 μ thick. Care was taken not to make the film so thin that the larger fat globules were distorted. Two slides were made from each sample. The slides were briefly examined under the microscope and were then chilled in a refrigerator to complete the solidification of the gelatin. The edges of the cover-glasses were then sealed with wax to prevent drying out of the film.

The slides were found to remain in good condition for at least a week. The solidified samples in the test-tubes showed very little change after holding in a refrigerator for 2–3 months. Portions of gel drawn from the tubes could be used for making up fresh slides if required. A small portion of the gel was placed on a slide, warmed just sufficiently to soften the gelatin, pressed out with a cover-glass and then cooled.

The samples were examined with a Leitz Ortholux microscope using objective 6L ($\times 45$) with Periplan ocular ($\times 12$). A preliminary examination of the slide was made with a 16 mm. objective to select an area of uniform distribution free from air bubbles and of suitable depth. The high-power objective was then turned in, the position of the stage remaining unaltered. The selection of the final field was thus made at random within the chosen area. All the globules in the field for the full depth of the preparation were recorded on a sheet of paper by drawing a diameter of each globule, using an Abbé camera lucida with a drawing board adjusted so as to give an apparent magnification of $\times 1000$. Globules with a diameter of less than 1 μ (1 mm. on the drawing board) were recorded as dots and were entered as 0·5 μ. The globules in four or five fields on each of two slides for each sample were measured, giving a total of at least 1000 globules per slide. The fields chosen were distributed as uniformly as possible over the preparation, e.g. one at the centre and three towards the edges equally spaced around the circumference. Globules under 2 μ were measured to the nearest 0·5 μ and those of diameter 2 μ and over to the nearest 1 μ. The numbers of globules of each size were totalled for each slide and percentage distributions were calculated.

RESULTS

A typical set of measurements for a sample of Vacreator-treated cream is given in Table 2. The percentage distribution of fat globules has been shown for each of the two slides separately to indicate the reproducibility of the results. Average values for the sample have been calculated from the combined totals for the two slides. The reproducibility of the results was usually of the same order as that in the example, provided at least 1000 globules were measured on each slide.

Table 2. *Size distribution of fat globules in a sample of Vacreator-treated cream*

Globule diameter (μ)	No. of globules: Slide 1, total of 4 fields	No. of globules: Slide 2, total of 4 fields	No. of globules: Total	Frequency (%): Slide 1	Frequency (%): Slide 2	Frequency (%): Total
0·5	706	611	1317	36	46	40
1	270	163	433	14 } 61	12 } 67	13 } 63
1·5	221	115	336	11	9	10
2	338	194	532	17 } 28	15 } 23	16 } 26
3	212	112	324	11	8	10
4	102	58	160	5	4	5
5	62	36	98	3 } 9·1	3 } 8·4	3 } 9·2
6	22	18	40	1·1	1·4	1·2
7	22	9	31	1·1	0·7	0·9
8	6	8	14	0·3 } 1·7	0·6 } 2·0	0·4 } 1·8
9	4	5	9	0·2	0·4	0·3
10	2	4	6	0·1	0·3	0·2
11	2	—	2	0·1	—	0·06
12	—	—	—	— } 0·2	— } 0	— } 0·1
13	2	—	2	0·1	—	0·06
Total	1971	1333	3304	—	—	—

While the method of expressing the results as a percentage of the number of globules is satisfactory for comparing one sample with another, it gives a rather misleading impression of the importance of the smallest globules. These may be sufficiently numerous to make up more than half the total number of globules but they actually contain only a small proportion of the fat. The distribution has therefore been recalculated as percentage weight of fat present, according to the method of Rahn (8). Where the results are to be expressed in this way it is important that the size classes, particularly in the small globules, should be as narrow as possible, otherwise the mean diameter taken for the size class may differ considerably from the true mean diameter. Lyons (6), who classed the smallest globules simply as 0–2 μ, took 1 μ as the mean diameter for this group but multiplied the calculated volumes by 4, on the assumption that this size class contained more globules of 1–2 μ diameter than of 0–1 μ diameter. Since in the present investigation globules under 2 μ in diameter have been measured to the nearest 0·5 μ such a correction is unnecessary.

The results given in Table 3 show that globules under 2 μ in diameter, although comprising 63% of the total number of globules (see Table 2), contained only 2·1% of the fat in the globules measured. Agreement between results for the two slides is satisfactory in the smaller globule sizes, but divergences in counts of large globules are greatly magnified when expressed as percentage weight of fat. This divergence in the higher size classes is unavoidable, owing to the small number of large globules present and to the great weight of fat compared with that in the smaller globules, carried by each of them. The presence of some large globules makes it essential to measure a greater number of globules than would be necessary, where (e.g. with milk) the distribution range is narrower. In order to obtain a more accurate estimation of the proportion of larger globules, Lyons made an additional count of 100 globules, 8 μ or over in diameter on each slide, for the purpose of checking the ratio found in the original count. This method has not been followed in the present investigation. Reliance has been placed on the creaming treatment to remove the largest globules and so reduce the error.

Results of determinations of size distribution of fat globules in raw cream and in Vacreator-treated and flash-pasteurized cream, produced from this raw cream, are given in Table 4. The Vacreator-treatment given was normal and at medium intensity, but the exit temperature was adjusted to 130° F., i.e. a little higher than usual. The flash-pasteurizer was a tandem beater-type machine. Cream was heated to 160° F. in the

Table 3. *Distribution of fat in globules in a sample of Vacreator-treated cream*

Globule diameter (μ)	Weight of 1 globule (mg. $\times 10^{-9}$)	Weight of all globules (mg. $\times 10^{-9}$)			Weight of globules as % of total weight		
		Slide 1	Slide 2	Total	Slide 1	Slide 2	Total
0·5	0·06	42·4	36·7	79	0·2	0·2	0·2
1·0	0·48	130	78·2	208	0·5 } 2·2	0·5 } 1·9	0·5 } 2·1
1·5	1·63	360	188	548	1·5	1·2	1·4
2	3·85	1,301	747	2,048	5	5	5
3	13·0	2,756	1,456	4,212	11 } 16	9 } 14	11 } 16
4	30·8	3,144	1,788	4,933	13	12	12
5	60·2	3,733	2,168	5,900	15 } 37	14 } 38	15 } 37
6	104	2,288	1,872	4,160	9	12	10
7	165	3,437	1,488	5,124	15	10	13
8	247	1,480	1,972	3,452	6 } 31	13 } 46	9 } 37
9	351	1,405	1,756	3,160	6	11	8
10	482	963	1,927	2,890	4	12	7
11	641	1,282	—	1,282	5	—	3
12	832	—	—	—	— } 14	— } 0	— } 8
13	1,058	2,117	—	2,117	9	—	5
Total		24,639	15,476	40,114	—	—	—

Table 4. *Distribution of fat in globules as percentage weight of fat in raw and pasteurized creams*

Globule diameter (μ)	Raw cream	Vacreated cream	Flash-preheated cream	Flash-pasteurized cream
0·5	0·1	0·5	0·1	0·05
1	0·4 } 1·9	1·2 } 4·0	0·3 } 1·0	0·2 } 1·05
1·5	1·4	2·3	0·6	0·8
2	4	5	2	2
3	17 } 21	11 } 16	7 } 9	8 } 10
4	26	16	12	11
5	23 } 61	16 } 43	12 } 33	11 } 33
6	12	11	9	11
7	5	7	7	5
8	2	7	6	4
9	2 } 10	3 } 19	6 } 22	4 } 15
10	1	2	3	2
11–15	3·7	4	8	9
>15	— } 6	10 } 18	23 } 35	27 } 41
Oiled off	2·4	3·9	3·9	4·7

first unit and to 200° F. in the second unit, and was sampled after cooling to 130° F. on a surface water cooler. Flash-preheated cream was passed through the second unit only of the flash-pasteurizer and was heated to 130° F.

The results show that Vacreator-treatment of cream causes a large increase in the amount of fat present as globules less than 2 μ in diameter, and also some increase in the amount present as large globules (> 10 μ). These increases occurred at the expense of the medium-sized globules (2–6 μ) which, in the raw cream, accounted for over 80% of the fat present. Flash-preheating and flash-pasteurization both caused some reduction

in the amount of fat present as small globules and a great increase in the amount present as large globules. The tendency to cause coalescence of fat globules was almost as great where the cream passed through only one unit of the pasteurizer for preheating as it was where it passed through both units for pasteurization.

Results of a further comparison are given in Table 5, which shows size distribution of globules in raw cream and in preheated creams produced from it in a flash-pasteurizer unit and in a plate heat-exchanger. In each case heating was carried to 130° F. as before. The effect of flash-preheating was similar to that in the previous experiment. The plate

Table 5. *Distribution of fat in globules as percentage weight of fat in raw and preheated creams*

Globule diameter (μ)	Raw cream		Flash-preheated cream		Plate-preheated cream	
0·5	0·1		0·05		0·05	
1	0·3	1·4	0·2	0·95	0·3	1·55
1·5	1·0		0·7		1·2	
2	4		3		5	
3	12	16	8	11	15	20
4	19		12		21	
5	20	52	13	38	21	55
6	13		13		13	
7	7		7		11	
8	3		4		4	
9	2	14	5	20	3	20
10	2		4		1	
11–15	3		20		3	
>15	10	17	4	30	—	4
Oiled off	3·7		6·4		0·9	

heat-exchanger, however, showed little tendency to cause either subdivision or coalescence of fat globules.

The results of five comparisons of fat globule distribution in raw and Vacreator-treated cream are given in Table 6. The raw creams consisted of factory-separated cream, which had been held for 1 or 2 days, mixed with a small proportion of farm-separated cream. The size distribution of fat globules in raw cream may vary considerably with the treatment the cream has received. The creams available for this experiment would be expected to show less aggregation of fat globules than farm-separated creams which had been subject to some degree of churning in transit to a commercial factory.

The results given in Table 6 confirm those presented earlier and show the tendency of Vacreator-treatment to cause subdivision of globules, thus considerably increasing the proportion of globules of less than 2 μ diameter. A slight tendency to cause aggregation of globules is also evident. These results are in agreement with the observation of Sargent (9) that Vacreator-treatment caused a decrease in the average diameter of globules, and provide an explanation of the increased fat losses which occur when Vacreator-treated cream is churned.

The tendency of beater-type flash-pasteurizers to cause aggregation of fat globules has been noted earlier (7) and has been studied in detail by Lyons (6). The photomicrograph in Pl. 1 (*d*) shows some of the large globules found in flash-pasteurized cream. Such large globules rarely occur in Vacreator-treated cream.

In addition to affecting the fat losses in churning, the difference in globule sizes affects the texture of the butter. As will be shown in the following section, this may be

accounted for by differences in microscopic structure of the butter which may be related to the method of pasteurization of the cream.

Table 6. *Size distribution of fat globules. Average for five raw and five Vacreated creams*

Globule diameter (μ)	Distribution as % no. of globules				Distribution as % weight of fat			
	Raw		Vacreated		Raw		Vacreated	
0·5	29		48		0·1		0·3	
1	13	53	17	76	0·4	1·6	0·8	2·9
1·5	10		11		1·1		1·8	
2	15	30	10	16	4	18	4	12
3	15		6		14		8	
4	9		4		19		11	
5	5	16	2	7	20	51	12	33
6	1·7		1		12		10	
7	0·5		0·6		6		9	
8	0·3	1·2	0·12	1·2	5	18	9	28
9	0·3		0·12		5		5	
10	0·08		0·12		2		5	
11–15	0·12	0·18	0·14	0·2	8	11	12	14
>15*	0·06		0·06		3		12	

* Includes 'oiled off' fat.

MICROSCOPIC EXAMINATION OF BUTTER

Method. A small piece of butter was detached from the sample with a needle and was placed in a cell 10 μ deep. A cover-glass was applied and pressed flat with another microscope slide.

It was found that if the temperature of the butter was too low when the slide was made the water droplets were severely distorted. A temperature of 16–18° C. was found suitable. The need to have the butter at the correct temperature was not mentioned by earlier workers on microscopy of butter, but has been remarked on by Hoyos(1) who specified a temperature of 14–16° C. for examination of water droplets in Fritz process butter.

Slides of butter from flash-pasteurized cream when examined under low power ($\times 50$) showed numerous white specks which proved to be fat aggregates. In slides of butter from Vacreator-treated cream the specks were much smaller. The appearance of the two types of butter under low-power magnification is shown in Pl. 2 (*a*) and (*b*). Examination of the white specks under higher power confirmed that they were masses of fat. In Pl. 2 (*d*) are shown several of these large aggregates with water droplets surrounding and overlying them. Globules or aggregates greater than 10 μ in diameter have been pressed out in making the slide. Their apparent diameter is thus considerably greater than their diameter in the original sample.

Butter from Vacreator-treated cream contained very few of these large aggregates (Pl. 2 (*c*)). Examination of the butter preparations under polarized light also showed that in butter from flash-pasteurized cream there were many intact fat globules over 10 μ in diameter (Pl. 2 (*f*)), while in butter from Vacreator-treated cream (Pl. 2 (*e*)) large globules could rarely be detected. It was difficult to find any sharply defined fat globules in preparations of butter from Vacreator-treated cream. If, however, the butter was diluted with butter oil according to the technique of King(2, 3) numerous small globules were visible.

The difference in microscopic structure of butter from flash-pasteurized and from

Vacreator-treated cream no doubt accounts for the difference in appearance and texture of the butters. The finer microscopic texture of butter from Vacreator-treated cream probably explains why its body and texture are generally preferred by graders to those of flash-pasteurized cream butter. The large fat-aggregates present in flash-pasteurized cream butter may be responsible for the 'mealiness' sometimes encountered with this butter.

SUMMARY

A method for determining the size distribution of fat globules in cream has been described. A thin film (20 μ) of the diluted cream on a microscope slide is preferred to a deeper preparation (0·1 mm.) in a counting chamber in which rise of fat globules to the upper surface is necessary for easy observation. The latter method is less convenient and can give too low an estimate of the proportion of the smallest globules (1 μ and under).

Cream should be diluted 1 in 50 and the concentration of gelatin in the dilution should be sufficient (4–6%) to give a rapid gel formation. The mixture should be cooled till the gelatin thickens before being transferred to the slide if it is to give the most uniform distribution of globules.

It is shown that Vacreator-treatment of cream causes a considerable increase in the proportion of fat present as globules less than 2 μ and a small increase in the proportion present as globules over 10 μ in diameter. Flash-pasteurization, on the other hand, causes a slight decrease in the proportion of fat present as small globules and a considerable increase in that present as large globules.

Butter from flash-pasteurized cream contains numerous large fat globules and fat aggregates, while butter from Vacreator-treated cream contains few large globules. It is suggested that the more uniform subdivision of fat in butter from Vacreator-treated cream explains why butter from Vacreator-treated cream is superior in texture to butter from flash-pasteurized cream.

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514. STUDIES ON A WASHED CURD VARIETY OF CHEESE

II. THE INFLUENCE OF VARIATION IN THE WASHING AND SCALDING PROCESSES

By JOAN WHITMORE

Dairy Department, The University, Reading

The characteristic flavour, body, and texture of washed curd types of cheese depend upon maintenance of a low acidity throughout the manufacturing process, which may be effected by using comparatively small amounts of starter, renneting immediately after adding the starter, replacing part of the whey with water during the scalding period, scalding to a fairly high temperature, and filling the curd into moulds immediately after pitching.

In particular, the washing and scalding procedures used for the manufacture of different cheese within the group vary considerably and are responsible for the production of their characteristic texture* properties. *Gouda* is generally washed either once or twice only, and is scalded to 98° F. In a recipe for *Danish Swiss* cheese Fisker (1) recommends a scald of 98–100° F. and washing four times by replacing one-quarter of the whey with water at each washing. The washing process should take 1 hr. to complete. The *Iowa Swiss* type is made by washing first with one-third of the whey substituted by water at 150° F., and then three or four times more with water at the same temperature to reach a scald of 106° F. (2). *Brick* cheese, which is not conventionally classed as a washed curd variety, was found (3) to be much improved when washed at the end of the scalding period with 25 lb. of water per 1000 lb. of milk.

In view of these variations in cheesemaking practice, the washing and scalding processes were studied while standardizing other points in the manufacture of this cheese. The manufacturing procedure described in a previous paper (4) was regularly followed during a 2-year experimental period. The effect of variations in the amounts of washing water used, temperatures of scald, and times of raising the scald were investigated.

(a) *Effect of varying amounts of washing water on cheese quality*

Certain terms have been used for the sake of brevity to describe the systems of washing. 'Total wash' indicates that the curd was washed four times. Using a 12 gal. batch, 3 gal. of whey for the first wash and of mixed whey and water for subsequent washes were replaced, in each case, with hot water. The temperature of the water was calculated to bring the curd to the desired scalding temperature. '$\frac{3}{4}$ wash' indicates only three and '$\frac{1}{2}$ wash' only two washes with hot water. The actual percentages of water present in the final mixture of whey and water are as follows:

Procedure	Amount of whey remaining in mixture	% water
$\frac{1}{4}$ wash	9 gal. whey in 12 gal.	25
$\frac{1}{2}$ wash	6$\frac{1}{2}$ gal. whey in 12 gal.	44
$\frac{3}{4}$ wash	5 gal. whey in 12 gal.	58
Total	3$\frac{1}{3}$ gal. whey in 12 gal.	70

* The term 'texture' is used to indicate qualities of openness, smoothness and firmness.

Experiments on the first eighty-three cheese were designed to standardize the manufacturing procedure. The effect of the washing process on the final texture qualities was recorded for each cheese and, since variations in texture are clearly distinguishable in this type of cheese, the grade was easy to judge. Quality of texture was assessed as follows: grade I, smooth and plastic; grade II, slightly rough; grade III, tough, dry; grade IV, coarse, granular, rough.

Analysis on this basis of all the results obtained yielded the figures given below:

Amount of washing	Grades of texture I	II	III	IV	No. of samples
Total	9	—	1	12	22
$\frac{3}{4}$	—	—	—	1	1
$\frac{1}{2}$	21	1	—	1	23
$\frac{1}{4}$	1	—	—	—	1
None	'Cheddar' type texture				1

Clearly the amount of washing the curd receives has a marked effect on the texture of the cheese. A wash replacing only one-half of the whey with hot water gave significantly better results than the total wash, and this procedure was therefore adopted as the standard for future experiments. It is in agreement with recipes given for the Iowa Swiss type of cheese (2), but not with that given by Fisker (1) for Danish Swiss. The $\frac{3}{4}$ and $\frac{1}{4}$ wash procedures were tried only once. The one control cheese made without washing amply demonstrates the effect of washing on the physical nature of the curd. It was short and crumbly and lacked the plasticity which is so characteristic of washed curd cheese.

One of the most important aspects of the washing process is its effect on the texture and body of the curd. A change in the physical properties becomes evident as soon as hot water is added: it contracts immediately and becomes more manageable, each piece of curd separating with a skin forming on the surface of every grain. This change in the nature of the curd is responsible for its quick matting properties and the development of a close texture and plastic body. These properties may well be largely responsible for the fact that when gas is formed during the ripening process it is held in the cheese in the form of bubbles. In this, washed curd cheese may be compared to Swiss cheese where the close texture is produced by high scalding temperature combined with violent and prolonged stirring. In both cases gas separates out in the form of bubbles.

In addition, washing has the effect of reducing the lactose content of the curd, thus maintaining a fairly high pH, so that the curd is suitable for the particular fermentations typical of washed curd cheese ripening. Low acidity in Swiss curd results in variations in the binding of the water, producing a plastic, soft, semi-transparent cheese, whereas a low pH produces brittleness and openness of texture and so should be avoided (5).

These experiments showed that the most desirable texture was obtained with a $\frac{1}{2}$ wash, while a tendency to toughness was encountered when excessive washing was used.

(*b*) *Effect of temperature and time of raising the scald on cheese quality*

The temperature of scald has an additional effect on the body and texture properties of the finished cheese. The scalding temperatures used in the manufacture of Dutch, Danish Swiss and Iowa Swiss types of cheese vary from 98 to 110° F.

By regulating the temperature of the washing water, the scald could be completed at

the same time as the washing process. A rapid rise of scald was therefore ensured without further recourse to steam heating, which was rarely necessary.

The results of the experiments are given in Table 1. The four grades previously used to describe body qualities were again employed, and eyehole quality was graded as: I, good; II, round but small; III, flat, irregular; IV, split, ragged; V, poor, cracked, pulled.

Table 1. *Relation between scalding procedure and texture and eyehole quality*

		Grading results in percentages of samples								
		Texture grade				Eyehole grade				
Procedure	Nos. of cheese	I	II	III	IV	I	II	III	IV	V
(1) Scalding temperature (° F.):										
98–100	4	100	0	0	0	0	0	50	50	0
102–104	29	66·6	4·8	2·4	26·2	42·5	7·5	14	22	14
105–107	24	75	12·5	0	12·5	25	25	25	25	0
110–112	11	54·6	9·1	18·2	18·2	27	9	0	27	37
114–115	11	36·4	9	36·4	18·2	36·4	27·2	0	36·4	0
120–125	4	0	0	100	0	0	50	0	50	0
(2) Scalding time (min.):										
8–14	4	100	0	0	0	75	25	0	0	0
14–18	25	70	9	17	4	32	24	8	28	8
19–23	23	45	9	12	34	39·7	12·9	4·3	21·5	21·6
23–24	12	36·4	9·1	9·1	45·4	55	0	22·5	22·5	0
26–30	11	45·5	0	18·2	36·2	18·2	9	27·3	36·4	9·1
31–40	8	100	0	0	0	0	0	0	50	50

Table 1 shows a distinct relation between scalding temperature and texture quality, the lower temperatures producing the most desirable properties in a washed curd cheese. The table also shows a higher proportion of good texture cheese in the shorter scalding time ranges; the incidence of grades III and IV was higher when scalding time exceeded 20 min.

Eyehole-forming properties were not very closely related to scalding temperature, although there is some indication of a more favourable range around 102–104° F. Variations in scalding time seemed more significant, and the data show a higher proportion of satisfactory eyehole cheese (grades I and II) within the shorter time ranges, with a corresponding rise in poor grade cheese when scalding time was prolonged, especially when more than 30 min.

On the basis of observations during these experiments, it was decided that a combination of a $\frac{1}{2}$ wash with a scald of 103° F. was most suitable for the manufacture of this type of cheese. The scalding and washing processes should not take longer than 25 min. Lower temperatures of scald help to produce the characteristic rather soft, plastic body and smooth, close texture. On the other hand, eyehole shape appeared to be affected more by the time range than by the temperature of scalding.

(c) *Note on the use of large size moulds*

The object of these experiments was to evolve a recipe for a washed curd variety of cheese which could be adopted for commercial manufacture in Britain, and it was thought that a larger cheese than the original 12 lb. size would be more popular in factory manufacture. A number of cheese were therefore made in which the curd was moulded in Leicester cheese moulds. A mould of this type contains all the curd from 30 gal. of milk and produces a round cheese of 16 in. diameter and $3\frac{1}{4}$ in. height.

Bulk heat-treated milk was used for these experiments. Varying temperatures of scald were investigated, and in all cases the half-washing system was used. $7\frac{1}{2}$ gal. of whey were removed and replaced with hot water and the process repeated once more.

From the results given in Table 2 it is evident that this method does not produce the type of cheese desired. The texture of all the cheese was dry and the body lacked plasticity. Eyehole formation was inferior and the flavour consistently below the grade of those made by the standard method.

Owing to the difficulty of waxing the cheese, bad cracking occurred in the coat and at the edges, and may have been responsible for excessive evaporation and consequently poor texture qualities. The higher temperatures of scald which were considered necessary for a sufficiently firm body to prevent deformation during ripening were probably an additional cause of rough texture.

Table 2. *Records of cheese made using* 30 *gal. of milk*

No.	Scalding time (min.)	Scalding temp. (° F.)	Texture	Eyeholes	Colour	Flavour
1	19	114	Coarse	Ragged	Bleached	Mild
2	22	114	Dry	Irregular, few	Good	Pleasant, but slightly bitter
3	20	110	Dry	Very poor, ragged	Bleached	Poor
4	20	110	Firm, not plastic	Good	Good	Fairly good, lactic
5	22	115	Dry, crumbly	Ragged	Bleached	'Hot'
6	25	115	Smooth	Ragged	Good	Mild, lactic
7	25	120	Dry	Very ragged	Bleached	Unpleasant, 'meaty'
8	29	120	Dry, crumbly	Too few, small	Bleached	Bitter
9	17	125	Tough, dry	Very few, small	Good	Sharp
10	15	125	Dry	Large and ragged	Good	Unpleasant, 'meaty'

The size of the cheese made handling difficult during the pressing period and on the shelves of the ripening room. In appearance the cheese was not nearly as attractive as the square 12 lb. variety.

SUMMARY

1. Eighty-three experimental washed curd cheese were made to investigate the effects of variations in the washing and scalding processes upon the qualities of texture and eyehole formation.

2. Texture was influenced markedly by the amount of washing received by the curd, and a 'half-wash' procedure which gave the most satisfactory results is described. Good texture was also favoured by low scalding temperatures (98–107° F.) and short scalding times (8–20 min.).

3. Eyehole formation was affected by scalding time (particularly exceeding 30 min.) rather than temperature.

4. The best combination of conditions appeared to be a 'half wash' with scalding at 103° F. for a period not exceeding 25 min.

5. Large cheese moulds (30 lb.) failed to yield a satisfactory product and their use is not recommended.

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515. CHEDDAR-CHEESE FLAVOUR AND ITS RELATION TO TYRAMINE PRODUCTION BY LACTIC ACID BACTERIA

By J. C. DACRE

The Dairy Research Institute (N.Z.), Palmerston North, New Zealand

Dahlberg & Kosikowsky (1, 2, 3, 4, 5, 6) investigated the influence of *Streptococcus faecalis*, used both as a pure culture starter and mixed with a commercial cheese starter, on the flavour of American Cheddar cheese, and observed a gradual increase in the tyramine (*p*-hydroxyphenylethylamine) content of the cheese as it ripened. There appeared to be a semi-logarithmic relationship between the tyramine content and the intensity of Cheddar flavour, a fact which led these workers to postulate some connexion between the compound and the development of cheese flavour. They pointed out, however (4), that 'tyramine was not the Cheddar flavour compound but served as a means of measuring bacterial activity that accentuated flavour production', and that the activity of the tyramine-producing bacteria did not account for all the cheese flavour.

Tyramine in cheese is evidently formed through the bacterial decarboxylation of the free tyrosine liberated from the cheese protein, a reaction brought about by several species of streptococci. Gale (7, 8) has stated that only Lancefield groups A, D and F streptococci, especially strains of *Str. faecalis*, possess the L(−)-tyrosine decarboxylase enzyme (but no other amino-acid decarboxylase) to a variable degree. The lactobacilli, as a group, have not yet been systematically examined for these specific amino-acid decarboxylases. Rodwell (9), however, briefly reported the isolation of a number of lactobacilli from horse-stomach contents, and found that washed suspensions of these organisms actively decarboxylated histidine, lysine and ornithine only. Recently, Lagerborg & Clapper (10) examined thirty-three strains of lactobacilli, mostly isolated from human saliva, and found that they were almost devoid of any amino-acid decarboxylase activity.

Hupfer, Sanders & Tittsler (11) presented a short preliminary report on tyramine production in American Cheddar cheese manufactured from both raw and pasteurized milk, and in various bacterial cultures. They found that a cheese starter containing a mixture of lactic streptococci and an enterococcus (*Str. faecalis*) strain, greatly increased tyramine production, but did not affect the average quality of the cheese. Some sixty strains of lactic acid bacteria were tested for tyramine production in milk containing added tyrosine. Only Lancefield group D streptococci and a group comprising *Lactobacillus brevis*, *L. buchneri* and *L. fermenti* gave tyramine values indicating the presence of the tyrosine decarboxylase enzyme system.

In view of the importance of deciding whether the work on tyramine would give a lead in the problem of the origin and mechanism of development of Cheddar cheese flavour, the effect of *Str. faecalis*, and of several other species of lactic acid bacteria isolated from cheese, on Cheddar cheese flavour was investigated. The tyramine content of the experimental cheese was also followed. The presence or absence of a L(−)-tyrosine decarboxylase in cultures of the various organisms was determined, and in the course of this work,

the opportunity was taken to carry out a general survey of the occurrence of this specific decarboxylase enzyme in the organisms comprising this Institute's collection of lactic acid bacteria.

MATERIALS

Bacterial cultures

(1) *Starter culture.* For the manufacture of the experimental cheeses, a starter consisting of equal proportions of two strains of *Str. cremoris* (HP and K) was used.

(2) *Cultures added to cheese milk.* The following cultures were investigated for their tyramine production and were added to cheese milk in order to determine their influence on cheese flavour:

Str. faecalis. National Collection of Type Cultures No. 6783.

L. plantarum (strain 1.1) and *L. brevis* (strain 1.2). Strains isolated and characterized by Sherwood (12), working in this Institute.

Leuconostoc. A strain repeatedly isolated from several Cheddar cheeses manufactured at this Institute during 1951.

(3) *Other cultures tested for tyramine production.* The following cultures were tested for their L(−)-tyrosine decarboxylase activity:

Streptococci (group N of Shattock and Mattick). Cheese-starter cultures maintained at 22° C. in daily subculture in sterilized skim-milk media.

L. brevis. Several strains, viz. American Type Culture Collection No. 367 (*L. pentoaceticus*), A.T.C.C. 8287, N.C.T.C. 6107, N.C.T.C. 4036 (*L. brassicae-fermentatae*) and *L. pentoaceticus* var. *Rudensis.*

Lactobacilli, Leuconostoc and *Micrococci.* Apart from some type cultures, all strains were isolated from New Zealand Cheddar cheese, some by Sherwood (12) and many others for this present work.

All the cultures were maintained in the laboratory in a litmus yeast skim-milk medium, sometimes with the addition of chalk.

EXPERIMENTAL METHODS AND RESULTS

(1) *Tyramine production and flavour development in Cheddar cheese*

Manufacture of experimental cheeses

Cheeses were manufactured in the Institute's factory in small experimental vats from 160 gallons of good quality flash-pasteurized milk. To the experimental vat containing 80 gallons of milk was added 1 l. of a clotted yeast skim-milk culture of the test organism, immediately after the addition of the mixed *Str. cremoris* starter, except in the case of cheese 1 where 18·2 l. of a 3-day skim-milk culture of *Str. faecalis* was added to the milk. A control cheese was manufactured in a second vat from the remaining 80 gallons of milk. Strict precautions were taken to avoid cross-infection from the experimental vat to the control. None of the added organisms had any significant effect on acid production in the vat, consequently all the experimental and control cheeses could be made approximately to the same time schedule and all reached approximately the same final acidity in the vat. The moisture, fat and salt contents and the pH values of the cheeses after 14 days in the curing room, indicated that the experimental and control cheeses, were all normal in composition. The cheeses were stored at 55° F. for the first 2 weeks and were then waxed and held at 45° F. and 80–82% relative humidity.

Tyramine analysis of experimental cheeses

The method developed by Kosikowsky & Dahlberg (3, 13) for the determination of free tyramine in cheese was used with some minor modifications of quantities. The colour intensity, produced on the addition of fresh sodium nitrite solution, was measured at 500 mμ using a Beckman Model DU Quartz spectrophotometer.

Tyramine production and its relation to flavour development

The experimental and control cheeses were examined at approximately monthly intervals, both for flavour development by a grading panel, and for tyramine content. The flavour results were all clear-cut. All the control cheeses developed normally and showed the mild, gradually increasing flavour characteristic of New Zealand Cheddar cheese made from high-quality pasteurized milk. Those cheeses containing added *Str. faecalis* (cheese nos. 1 and 2) showed a very marked increase in flavour intensity, especially during the first few months of ripening. Cheese 1 was especially strong in flavour after 2 months, but some observers considered that the flavour was not quite normal. As the ripening progressed, the differences in flavour between the *Str. faecalis* cheeses and the controls tended to decrease. The cheeses containing added *L. plantarum* (no. 3), the *Leuconostoc* strain (no. 5) and the mixed *L. plantarum*/*Leuconostoc* strain (no. 4) all showed a similar enhanced Cheddar flavour as compared with the controls, up to 6 months of ripening. The *L. brevis* cheese (no. 6) produced a small initial flavour increase when compared with the control, but after 2 months the flavour became distinctly 'yeasty' or 'fermented' in character.

Table 1 gives the corresponding tyramine analysis figures for the experimental cheeses. All the control cheeses gave values for tyramine ranging from 1·5 to 13 μg./g. of cheese.

Table 1. *Tyramine values (μg./g. of cheese) for the experimental cheeses*

No. of cheese ...	1	2	3	4	5	6
Added organism ...	*Str. faecalis*	*Str. faecalis*	*L. plantarum*	*L. plantarum*/*Leuconostoc* strain	*Leuconostoc* strain	*L. brevis*
Ripening period						
2 months	460	382	13	—	6·5	70·5
4 months	—	430	—	—	—	345
5 months	—	—	20	23	8	—
7 months	775	—	—	—	—	—

Tyramine production and increased Cheddar flavour development was found in the *Str. faecalis* cheeses as expected on the basis of the findings of Dahlberg & Kosikowsky (4). Although there was an enhanced flavour in the cheeses to which *L. plantarum* and *Leuconostoc* had been added, both singly and in admixture, the amounts of tyramine present were very similar to those in the control cheeses. A large amount of tyramine was formed in the cheese to which *L. brevis* had been added, but the flavour that developed was 'yeasty' and 'fermented', in agreement with the findings of Sherwood (12) and Tittsler, Sanders, Lochry & Sager (14) using this species.

Although the experiments were few in number, the results were obviously quite definite enough to show that there is no direct connexion between the formation of tyramine, by the bacterial decarboxylation of tyrosine, in Cheddar cheese and the development of cheese flavour.

(2) *Decarboxylase activities of the lactic acid bacteria*

Method of determination of decarboxylase activity

The differential pH method devised by Gale, and reported by Sharpe (15), was followed. Tubes of the basic medium and the same medium plus 0·5% of L(−)-tyrosine, inoculated with one loopful of a culture of the organism grown for 24 hr. in a broth medium devised by Hunter (16), were incubated at the appropriate temperature (22, 30 or 37° C.) for varying periods. The differences in pH units between the two types of media were determined using a Beckman glass electrode. If the organism produced a specific amino-acid decarboxylase, the formation of the amine caused a rise in the pH of the amino-acid enriched medium. In the case of tyrosine, the difference in pH units between the inoculated basic medium, and that plus tyrosine, was taken as a measure of the tyrosine decarboxylase activity.

Results for the lactic acid bacteria

Some 100 different species and strains of lactic acid producing bacteria (streptococci (Shattock and Mattick group N), 17%; micrococci, 10%; leuconostoc, 9%; lactobacilli, 64%), when tested by Gale's differential pH method, showed the absence of a tyrosine decarboxylase enzyme system. Cultures of *L. buchneri* A.T.C.C. 4005 and *L. fermenti* A.T.C.C. 9338 also gave negative results (cf. Hupfer *et al.* (11)). Strains of *L. brevis* were the only organisms found to produce a large difference in the pH values during growth. Table 2 gives the average pH values for the basic medium (B) and the 0·5% tyrosine medium (T) with strains of this species. The results indicate that this species elaborates the specific L(−)-tyrosine decarboxylase enzyme.

Tests with one strain of *L. brevis* (strain 1.2) failed to disclose any decarboxylase activity when the L-isomers of lysine, ornithine, histidine, glutamic acid, valine, alanine, leucine, proline, tryptophane and DL-methionine (1·0%) were substituted for tyrosine in the basic medium.

Table 2. *Tyrosine decarboxylase activity of* Lactobacillus brevis *strains as shown by* pH *measurements*

		Days of incubation at 30° C.						
L. brevis strain	Medium	1	2	4	7	10	14	21
1·2*	B	6·38	5·10	4·73	—	4·72	4·73	4·73
	T	6·52	6·20	6·55	—	6·50	6·46	6·50
A.T.C.C. 367	B	—	6·09	4·74	—	4·56	4·57	4·50
	T	—	6·31	6·21	5·60	6·04	6·30	7·09
N.C.T.C. 4036	B	—	6·10	5·28	4·86	4·73	—	4·80
	T	—	6·33	5·74	5·50	5·47	6·12	6·10
Str. faecalis	B	4·66	4·66	4·67	—	—	—	—
N.C.T.C. 6783	T	6·15	6·77	7·76	—	—	—	—

* Strains N.C.T.C. 6107, A.T.C.C. 8287 and *L. pentoaceticus* var. *Rudensis* gave very similar figures.

Paper partition chromatographic analysis of the culture filtrates of the Lactobacillus brevis *strains*

To confirm the production of tyramine from tyrosine by all the strains of *L. brevis*, the cultures grown in Sharpe's medium at 30° C. for the differential pH measurements were used. When the tyrosine in the medium appeared to have been almost completely

metabolized by the inoculated organism (after 10–14 days), the culture was filtered through a small Seitz filter and a few drops of the culture filtrate applied as a spot to the corner of a filter-paper sheet for two-dimensional paper partition chromatographic analysis by the methods of Consden, Gordon & Martin (17), Woiwod (18) and Bremner & Kenten (19). Chromatograms prepared from all the *L. brevis* cultures containing added tyrosine showed the complete absence of the amino-acid, together with the appearance of a new spot, identified in the usual way with tyramine.

The isolation of tyramine and the quantitative decarboxylation of tyrosine by Lactobacillus brevis

For the isolation and identification of tyramine, a flask containing 100 ml. of Sharpe's medium with the addition of 0·5% of L(−)-tyrosine, was inoculated with *L. brevis* (strain 1.2) and the flask was immediately connected, by means of a sterile glass bridge and rubber bungs, to a receiver containing a measured volume of 0·1 N-$Ba(OH)_2$. The culture was incubated at 30° C. At intervals, the residual unchanged baryta was determined by titration with 0·1 N oxalic acid. From the titration results the volume of carbon dioxide produced was calculated. Appropriate blank controls were also run. From one of the flasks, after 21 days' incubation, the unmetabolized tyrosine was separated by filtration, and the tyramine in the culture filtrate was identified as the dibenzoyl derivative after the method of Gale (7).

Table 3 sets out the volumes of CO_2 produced, and the weights of tyrosine metabolized by *L. brevis* (strain 1.2). The results indicate a strictly quantitative decarboxylation of tyrosine to tyramine and CO_2 after allowing for the CO_2 produced during the normal sugar metabolism of the organism.

Table 3. *Tyrosine degradation and carbon dioxide production by* Lactobacillus brevis (*strain* 1.2)

Medium	Incubation period (days)	Total vol. CO_2 evolved (ml.)	Vol. CO_2 from tyrosine used (ml.)	Wt. of tyrosine used (g.)	Theoretical vol. CO_2 from tyrosine used (ml.)
Tyrosine (0·5%)	14	56·8	30·6	0·25	30·9
Tyrosine (0·5%)	21	60·6	34·0	0·29	35·8
Tyrosine (0·5%)	21	61·8	35·2	0·28	34·6
Basic	14	26·2	—	—	—
Basic	21	26·6	—	—	—

DISCUSSION

The results detailed above indicate, from more than one point of view, that tyramine has little if any connexion with the substance or substances responsible for the typical flavour of Cheddar cheese. It is true that when *Str. faecalis* is present in a cheese, there is a certain parallelism between the formation of tyramine and the development of cheese flavour, but this parallelism does not hold when other species of cheese bacteria are added to cheese milk. *L. brevis*, one of the few species of lactic acid bacteria which forms a significant proportion of tyramine by the decarboxylation of tyrosine, produces a definitely undesirable 'yeasty' flavour in the cheese, while certain other lactobacilli which form very little, if any, tyramine in cheese, always enhance cheese flavour more significantly than most other organisms. Even with *Str. faecalis*, there was a doubt in the present work whether the flavour produced was an entirely normal Cheddar cheese flavour.

In the present experiments, strains of *L. plantarum* (see also Tittsler *et al.* (14)), *Str. faecalis* and *Leuconostoc*, were all found to be capable of increasing the rate of flavour development in cheese. Alford & Frazier (20) found that *Micrococcus freudenreichii* strains, isolated from American Cheddar cheese, also had a beneficial effect on flavour. Since the power to form tyramine is not a common property of all these species, the indications are that tyramine formation does not run parallel with flavour development. Obviously, tyramine formation cannot be used as an index of curing in the early days of ripening as suggested by Dahlberg & Kosikowsky (5).

In this work, an examination of many lactic acid bacteria belonging to the family Lactobacteriaceae Orla-Jensen (21), gave the result that the tyrosine decarboxylase enzyme system was present in only one species. None of the group N streptococci (of Shattock and Mattick) and *Leuconostoc* strains tested, were found to possess the specific enzyme, and of the Lactobacilleae Winslow *et al.*, excluding the Propionibacteria and Butyribacteria, which were not examined, only *L. brevis* showed a significant tyramine-producing power. These results emphasize the improbability of a connexion between tyramine production and Cheddar cheese flavour.

SUMMARY

1. Cultures of *Str. faecalis*, *L. plantarum* and a strain of *Leuconostoc*, when added to cheese milk, all brought about an increase in Cheddar flavour intensity in the resultant cheese.

2. Examination of the cheeses for tyramine content showed that the formation of the amine bore no relationship to the increases in flavour intensity. The production of tyramine appeared to be merely incidental in the cheese-ripening process.

3. A survey among the lactic acid bacteria disclosed only one species containing a significant amount of the L(−)-tyrosine decarboxylase enzyme—*L. brevis*. This species added to cheese milk causes an objectionable 'yeasty' flavour in the final cheese.

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REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION A. PHYSIOLOGY OF DAIRY CATTLE

I. REPRODUCTION AND LACTATION

The main object of this article is to give a brief account of the research work which has been published on reproduction and lactation in dairy cattle since the end of 1949 when the previous review of the present series was prepared (1). There is no intention to trace the history or development of any aspect of the subject, but simply to indicate the main ways in which knowledge has advanced in the past two years. Frequent reference is made to other reviews which have been published recently and which, since they deal with a more limited field, include details of earlier work.

REPRODUCTION

In the past few years, partly owing to the development of artificial insemination with all the additional evidence which can thereby be attained, increasing attention has been paid to problems associated with fertility in the dairy herd. An important paper published by Foot & Ridler (2) is concerned with the herd of the National Institute for Research in Dairying during the ten years 1937–47. Of 627 cows and heifers which were served at least once during that period 53·6% conceived after the first service, 16·9% required two services for conception to occur, 9·1% required three, 5·9% required four and 2·6% conceived only after the fifth or later service, a total of 88%. Some of the remaining 12% had been disposed of after only two ineffective services, and from a detailed consideration of the records the authors concluded that absolute sterility occurred in less than 5% of the herd each year, and that there is a danger of over-estimating the incidence of true sterility. It is clear, however, that much could be gained if the conception rate after only one service could be substantially increased, for it is seldom convenient or economic to wait until after the fourth or fifth service to decide whether a cow should be culled. In an article by Hammond (3) attention is drawn to the fact that on the average only about 64% of cows in many districts of England and Wales conceive after one service or insemination. He points out that the failure of cows with apparently normal reproductive tracts to conceive may result from some slight abnormality in the oestrogen-progesterone level in the blood. Excess of oestrogens may cause too rapid a passage of the ova down the tubes which would tend to prevent the ova from being fertilized, whereas excess progesterone may cause them, if fertilized, to reach the uterus before it is in a suitable state to receive them so that they would degenerate there. Certainly Laing (5) found for two groups of twenty-four heifers that degenerated fertilized ova and early embryos did occur in animals in which the reproductive tracts appeared to be normal, and from work by Hansel & Trimberger (4) it is suggested that lack of progesterone at the normal time of ovulation may be the cause of various types of infertility in cows. Two German workers (6) have described a type of sterility they encountered in cattle which they believed to be caused by an excessive quantity of oestrogenic substances in the pasture the cattle were grazing. It was thought that the oestrogen, which was estimated to amount to as much as 2 g./kg. dry matter, came from dung which contained the urine of pregnant cattle.

Durrell has continued his work on sterility in Canadian cattle. His recent publication (7) records data collected for 114 herds comprising over 5000 cattle and including Ayrshires, Holstein-Friesians, Jerseys, Guernseys, Dairy Shorthorns and Brown Swiss cattle. Sterility occurred in 71% of the herds and in about 10% of the animals and affected all breeds. It was permanent in less than 1% of the animals, most of it being attributed to temporary causes such as anoestrus or delayed conception. Malnutrition was believed to be a direct or indirect cause in almost half the cows which showed sterility. Some of the nutritional factors which can influence fertility have been discussed by Hignett & Hignett (8, 9), whose work on the effect of

calcium and phosphorus intake has been much discussed in the past two years. They suggest that the dairy cow to-day in Britain is consuming much more calcium than in pre-war years, and point out that excess calcium is well known to interfere with the utilization of phosphorus. They concluded from results for 802 cows and heifers that when P_2O_5 intake was high a low intake of CaO was often associated with low fertility and a high CaO intake with high fertility. It appeared that for high fertility the P_2O_5 intake should be greater than the generally accepted minimum levels of 23 g. for maintenance and 19 g. for each gallon of milk. In fact, from subsequent work (10) they inferred that for satisfactory fertility in housed heifers a minimum daily intake of 50 g. P_2O_5 appeared to be necessary. The authors stress (9) that they regard their earlier results as little more than 'pointers', and that much more work on a wider scale is required; they warn the reader against exaggerating the importance of the influence of calcium and phosphorus intakes in producing infertility, and draw attention to the probable importance of vitamin D (11). An interesting semi-popular article, founded on some of Hignett's work, has been published in the *British Agricultural Bulletin* (12). It contains a diagram which illustrates what a large number of factors may be involved. The fact that sunlight and vitamin D may be of considerable importance has been stressed by Abrams (13, 14, 15), who suggests that many of Hignett's observations may have resulted not so much from phosphorus deficiency in the diet as from an insufficiency of vitamin D which would result in diminished mineral absorption and utilization. He indicates (15) that the data assumed by Hignett & Hignett (10) to show a correlation between conception rates and the Ca/P ratio of the diet could possibly be interpreted as showing a correlation between time of insemination (i.e. season of year, amount of sunlight) and conception rate. The reader should refer to the original papers for a full appreciation of the arguments involved. For further details on the general subject of nutrition and fertility reference should be made to an account of a conference on the subject at which papers were read by Hammond on the physiology of reproduction in relation to nutrition (16), by Edwards on variations in fertility levels in bovines (17), by Folley on female fertility (18) and by Walton on spermatogenesis (19). Some of the purely physiological aspects of fertility have also been reviewed recently by Hancock (20). Other publications on sterility in cattle which have appeared in the last two years include papers by Engel (21) from Australia, by Schreiber (22) from Württemburg and by Van den Burg (23) from Holland. Asdell (24) has recorded that for a total of nearly three million cows for which records for 1932–49 were available in the United States, an average of 16·8% were culled each year, the chief reasons for culling being low productivity, sterility and udder troubles.

In a brief letter published in 1950, Edwards (25) has shown the importance of the lengths of the interval between calving and the first service thereafter on the conception rate. From records for nearly 12,000 cows in England and Wales he found that when the interval was 30 days or less the conception rate at first mating was only 48·3%. When the interval was 60 days the conception rate rose to 62·5%, and when it was 90 days the rate was 73·1%. With intervals of 90–180 days the rate remained at 70–73%. The increase in conception rate up to 90 days was highly significant, and indicates that the reduced fertility sometimes attributed to season of the year (for example, in January and February) may in fact be caused by a shortening at that time of the interval between calving and service. Much the same trend was found for almost 600 cows representing five different breeds in Illinois (26), where the results for 1674 pregnancies showed that when the post-partum interval to first service was only 1–20 days only 35% of services resulted in conception. This value increased to a maximum of 58 when the interval was 100–120 days. It would seem (27) that for reasonably high conception rates to be obtained there should be an interval of at least 50 days between calving and mating, and even greater intervals for maximum rates. Lewis & Horwood (28, 29), in Michigan, found that fewer services were required for conception by Holstein-Friesians than by some of the other breeds which had a shorter average calving interval.

The length of time which elapses between calving and the first oestrus has been studied recently by Herman & Edmondson (30) at Missouri, who analysed records for 968 parturitions

in 347 cows with normal pregnancies. The average interval was 57 days, with a standard deviation of 28 days. It seemed to be unaffected by the season of the year or by the average daily milk production either for the whole lactation or in the interval itself. Age had an effect, the interval being about 75 days for first-calf heifers (1½–2½ years old), 50–60 days for cows from 2½ to 7 years old and 60–90 days for older cows. The average value of 57 days just referred to is very close to that of 58·3 obtained in Minnesota (31), from data for 123 Holstein-Friesian cows in which individual values ranged from 20 to 135 days. The predictability of breeding efficiency in dairy cattle from one year to another has been studied at Kentucky by Olds & Seath (32), who found that nearly 55% of over 6000 cows required the same number of services in each of two years. According to Casida & Wisnicky (33), injection of 20 mg. diethylstilboestrol dipropionate within 9 days after calving failed to produce any significant alteration in the time which elapsed between calving and the first oestrus which averaged 60 days for the Holsteins used in their work.

Some years ago it was suggested that administration of ascorbic acid increased conception rate in cows of low fertility. Workers at Wisconsin (34), who claim that the earlier work was not well controlled, have now made a detailed investigation of the response of low-fertility cows to ascorbic acid, and since trichlorobutyl alcohol is known to increase the level of ascorbic acid in the plasma of cattle, this substance was also administered. They found that the percentage of cows with normal embryos at 34 days after breeding was 26 in forty-three control cows and 30 in forty-five treated cows, and that the treatment with ascorbic acid and trichlorobutyl alcohol did not increase the conception rate in cows of low fertility.

Hansel & Trimberger (35), at Cornell, have made experiments to determine whether the secretion of the luteinizing hormone of the anterior pituitary is under nervous control in cattle. The time of ovulation was determined in five heifers in a control oestrous period, and in a subsequent period in which atropine, which is known to block the release of the luteinizing hormone, was administered. Ovulation was delayed by 24–66 hr. by the treatment. When, however, both atropine and chorionic gonadotropin (as a source of the luteinizing hormone) were given in a similar well-controlled test, ovulation was not postponed. They concluded that a neural factor is involved in the release of the hormone and ovulation in dairy cattle. Since the time at which the chorio-allantoic membrane becomes attached to the uterine wall may be a critical stage in the development of the bovine embryo, Melton, Berry & Butler (36) have made a detailed study of the development of the embryo as it becomes attached to the maternal tissue. Twenty-four cows and heifers were used. They were killed at various intervals from 676 to 927 hr. after ovulation. The description of the work includes a series of photographs illustrating some of the changes which occur over a period of several weeks as the attachment gradually takes place. There is also a photograph showing eighteen embryos ranging in age from 676 to 927 hr. from time of ovulation; their weights and measurements are given.

The rate of development of the bovine foetus in 113 pregnancies covering four different breeds has been discussed by Swett, Matthews & Fohrman (37). The paper contains so much of importance on the foetus that it cannot be adequately reviewed in the space available here. Braude & Walker (38) have recorded and analysed the weights and a series of thirteen body measurements of seventy-four newly born Shorthorn calves. They found that while a cow was still growing the size of her offspring increased up to the third calf, but thereafter no direct relation was found between the size of a mature cow and her calf. The paper includes heifer calf mortality rates for the Dairy Shorthorn herd at the National Institute for Research in Dairying from 1924 to 1947. The total rate was 16·3%, of which 11·5% was due to prenatal losses and 4·8% to postnatal losses during the first 6 months post-partum. Bianca (39) has studied the weights of heifers throughout pregnancy. The uterus and products of conception were removed from ninety-nine slaughtered heifers and weighed. The weight of the uterus of twelve non-pregnant cows and heifers was also obtained. From the results and a knowledge of the age of the foetuses, a curve was drawn showing how the weight of the products of conception increased with time. With the aid of this curve the actual live weights of heifers could be estimated at any stage of pregnancy. In this

connexion, it is of interest to note that according to the results of Morrison (40) for over 200 heifers the body weight on the day of oestrus tends to be 3% less than on the previous day.

The length of the gestation period has continued to be a subject of research during the past two years. Braude & Walker (38) found that for 230 Dairy Shorthorn cows the average was 285·6 days. Calves carried longer were heavier at birth, the average increase being about 0·9 lb. per day of gestation period. The mean birth weight of the calves was 81·7 lb. Bull calves were carried longer than heifer calves by about 1·68 days, and were heavier at birth by about 3·39 lb. For Holstein-Friesians in Wisconsin, Jafar, Chapman & Casida (41) found the mean gestation period for 384 pregnancies to be 278·2 days with a standard deviation of 4·8 days. On the average, male calves were carried nearly 2 days longer than females. In this work the season of the year in which calving occurred appeared to have no effect on the length of the gestation period, whereas in a short abstract on recent work by Alexander (42) at Illinois, gestation periods preceding calvings in the summer months are claimed to have been somewhat shorter than for other seasons. In Jafar's work, as in that of Braude & Walker, there was a close positive correlation between the length of gestation and the birth weight of the calf. Another detailed study of the duration of pregnancy and factors which affect it has been described by Brakel, Rife & Salisbury (43) from Ohio. For a total of 1256 gestations the average was 279·6 days, the individual figures ranging from 245 days for a Jersey cow to 305 days for an Ayrshire. The means for the five main breeds were Brown Swiss 288·4, Guernsey 282·7, Friesian 278·6, Ayrshire 278·2 and Jersey 277·9 days; the means for the last three did not significantly differ from one another. The gestations preceding the birth of male calves (631 in all) averaged 0·77 day longer than for 625 gestations preceding the birth of female calves. The periods for spring-calving cows averaged 2·07 days longer than those for autumn-calving cows. Jasper (44) reports from California the instance of a Holstein-Friesian herd with about 250 cows in which the sporadic appearance of prolonged gestation has become a problem. In more than thirty pregnancies in 2½ years the normal gestation period has been exceeded by about 20–88 days, the calves at birth weighing from 105 to 168 lb. as compared with 90–110 lb. for normal calves. None of them has lived more than a few hours. The physiological causes for the postponement of parturition are not known, but it has been shown that the condition is inherited as a simple recessive character. Detailed records kept at Bangalore by Lazarus & Anantakrishnan (45) for a large number of pregnancies shows that the average lengths of the periods for Sindhi, Gir and Ayrshire-Zebu crosses of different types were 283, 285 and 280 days. Again, male calves were carried longer than heifer calves in all the breeds. The season of the year in which calving occurred did not appear to influence the length of the period, and cows showing one or more short gestations also exhibited gestations longer than the average. The effect of gestation on persistency of lactation has been studied by Erb, Goodwin, Morrison & Shaw (46). The rate of decline in yield was three to four times faster from 181 to 223 days of pregnancy than it was from 101 to 180 days, but in non-pregnant cows the rate of decline in lactation showed little change until it slowed down considerably after 330 days. The time at which inhibition started as a result of pregnancy appeared to be unaffected by age, yield, season of the year, calving interval up to 18 months or breed.

Laing (47) has continued his studies on oestrous behaviour and ovulation in the cow. From observations on forty-eight oestrous periods in forty cows during May, June and July of two successive years he found that the average duration of pro-oestrus, oestrus and metoestrus was 14·9, 12·4 and 6·8 hr. The ranges for all three periods were very wide, 0–72, 8–24 and 0–40 hr. respectively. Laing discusses the effect of abnormalities of oestrous behaviour on fertility, and points out that, in general, animals are inseminated at a time when the sperm can live until ovulation occurs or can reach the Fallopian tube before the ovum dies, but where the range of duration of oestrus can vary so much and where pro-oestrus and metoestrus may at times be indistinguishable from true oestrus, insemination may well occur at the wrong time. He points out that it is generally in the summer months when oestrous periods are long that fertility is sometimes diminished. Another cause of delay in conception which Laing discusses briefly is the occurrence of ovulation without oestrus or with only brief and ill-defined oestrous behaviour.

Further data on the length of the oestrous cycle in cattle has been published in the past two years. Roark & Herman (48), in a detailed and well-illustrated research bulletin on the physiology and histology of the bovine oestrous cycle, state that the average length of 504 oestrous cycles was 21·4 days, with a range of 11–35 days, 80% of the cycles being within 18–24 days. Olds & Seath (49) have obtained data for nearly 4000 oestrous cycles involving 278 cows. The mean cycle length was 32·4 days with a range of 1–549 days. Nearly 12% of the periods were 53 days or longer. The modal length was 22 days, and this represented about 12% of the periods, and to get a mean of 22 days all intervals of 36 days or more had to be omitted. When this was done about 83% of the intervals fell between 17 and 26 days. Studies on oestrus in the cow have also been made at Minnesota by Masuda, Onishi & Kudo (31). They found the average length of the oestrous cycle for 1134 Holstein cows to be 22·5 days. The average duration of oestrus was 21·6 hr., and on an average ovulation occurred 29 hr. after the onset of heat. In seventy-five of seventy-seven cows ovulation took place after the end of oestrus. They found that the conception rate was highest (93·3%) during a 4 hr. period covering the last hour of oestrus and the 3 hr. following it. Asdell (50) has stated that the mode of the oestrous cycle in heifers is 20 days and for mature cows 21 days. He found that ovulation generally occurs 13–14 hr. after the end of heat, and that heat induced in ovariectomized heifers by oestrogen lasts for the normal period despite continued injections. Hormone therapy in the treatment of sterility in cattle has been discussed also by Sporri (51) and Brigatti (52). Brigatti's experiments involved 146 cows which, owing to hypo-ovarianism or to persistent corpora lutea, had never come on heat. When treated with either synthetic or natural oestrogens 123 of them came on heat and later conceived. According to the author, synthetic oestrogens caused the formation of ovarian cysts in eight of the animals. Meites, Webster, Young, Thorp & Hatch (53), at Michigan Experimental Station, have experimented with pregnant goats to verify the need for the corpora lutea of pregnancy and to determine the minimal daily dose of progesterone required to maintain gestation after excision of the corpora lutea. It was found that after removal of all the corpora lutea, subcutaneous injections of 10 mg. progesterone daily in the latter part of pregnancy were sufficient to maintain the pregnancy. The effect of progesterone on the follicular development and the onset of oestrus has been studied by Ulberg, Christian & Casida (54). They found that injections of 50 mg. daily suppressed heat and ovulation if begun before the onset of heat, but inhibition of most of the follicular development occurred only when the injections were started on the 15th day of the cycle. It is suggested that progesterone prevents the action of the luteinizing hormone on the ovary. The interval which elapsed between the end of the injections and the beginning of heat decreased as the dosage level decreased.

For an account of a recent cytological study of the anterior pituitary in relation to the oestrous cycle reference should be made to a short paper by Paredis, Morgan & McNutt (55). Work on the cytological changes which occur in the cervical mucosa of the cow throughout the oestrous cycle (56) and on the cyclical changes which occur in the uterine mucosa and vagina of the goat (57) has also been described recently.

Reliable diagnosis of pregnancy in cattle is a problem which has not yet been solved. Foot & Ridler (2) have discussed the accuracy of diagnosis by palpation per rectum made mainly about 2 or 3 months after service. Of 213 animals described as pregnant only four were later found to be non-pregnant, and of the thirty-one animals described as not pregnant or probably not pregnant, six were later found to be in calf. In a group of thirty-nine animals examined less than 60 days after service only one was diagnosed incorrectly. In a brief letter, Hancock (58) has described his method of diagnosing pregnancy by palpation of the uterus and foetal membranes. He claims that he can make a correct diagnosis in 90% of heifers at 35 days and in 90% of cows at 40 days. Glover & Scott Blair (59) are making a study of the rheological properties of the cervical secretions. The preliminary results recorded in this brief account are to be extended to determine whether a reliable early test for bovine pregnancy or a test for sterility can be based on the rheological properties of the cervical secretions.

The chemical composition of bovine spermatozoa has been studied by Porter, Shankman &

Melampy (60) who found that the dried sperm from semen samples of seven bulls contained 1·2% total lipid, 1·1% ash, 2·3% phosphorus and 16–17% nitrogen. Seventeen of the amino-acids were estimated by microbiological methods. Arginine was the most abundant amino-acid present. Sarkar, Luecke & Duncan (61) have determined eleven of the amino-acids present in the spermatozoa and seminal plasma. Except for methionine, threonine and valine, the results for spermatozoa recorded by Porter *et al.* do not agree very closely with those obtained by Sarkar and his colleagues. In experiments by Rothschild (62) the activity of spermatozoa in bull semen has been assessed by measuring the frequency of impedance changes in the semen. It has been shown that the small amount of electrical treatment involved is harmless, so that measurements can be made on samples of semen which are subsequently to be used in artificial insemination. In later experiments the same author (63) has described a method whereby a Spekker absorptiometer can be used to determine the number of spermatozoa in a sample of diluted bull semen.

Certain instances of impotence in bulls were concluded by Sandstedt (64) to be caused by phosphorus deficiency. Of thirty-two impotent bulls fourteen responded to a higher level of phosphorus in the diet.

A detailed and informative discussion of causes of sterility in bulls in southern England has been published in four papers by Haq (65) from the Veterinary Investigation Laboratory, Reading. Partial or complete sterility in nineteen of thirty-three bulls examined was attributed to testicular degeneration, and in four of them to testicular hypoplasia. Causes in nine of the remaining bulls were balanites, paunchiness and faulty erection. A description of the general findings for each of these defects is given under such headings as history, behaviour at service, semen examination, clinical examination and autopsy. The functional anatomy of the prostate gland of thirty-two cattle of various breeds and of various ages has been discussed by Schenker (66).

One of the most important developments in recent years, and one which may have far-reaching effects in the future, has been concerned with the transplantation of fertilized ova from one cow to another, a process which would enable the eggs of cows of high genetic quality to be incubated in cows of inferior worth. Some of the problems involved have been indicated in an article by Hammond (67), in whose research school so much valuable work has already been done on the subject. Hammond points out that the first problem is to be able to increase the number of eggs shed by the cow so that the progeny of cows of good breeding value can be increased as is done for bulls of good quality by artificial insemination. He outlines four methods for attaining this end. They involve hormone treatment which in one or two of the methods is followed by squeezing out the corpus luteum. Under the best conditions the ovaries are not over-stimulated, six to twenty-five eggs may be shed and most of them fertilized. Methods of inducing multiple ovulation have been described in greater detail recently by Rowson (68), one of Hammond's colleagues at Cambridge. The next problem is to obtain the fertilized eggs from cows without having to slaughter them. For this purpose Rowson & Dowling (69) have devised an apparatus which can be inserted through the cervix into the upper end of the uterus 5 days after heat, and through which the eggs can be flushed out with cow-blood serum, a medium which does not injure the eggs. The eggs can then be inserted into the upper end of the uterus of the host cow about 5 days after heat by the same method as is used in artificial insemination. Further details can be obtained from a valuable paper by Dowling (70), in which he has described the extensive work he has done at Cambridge on problems associated with the transplantation of fertilized ova. The process met with considerable success when applied to rabbits, but when Dowling wrote his paper it had not so far been successful with cows. Nevertheless, much progress has been made, and the process should ultimately be practicable with cows. A popular article on the subject has been published by Rowson (71), and accounts of progress made in America on isolating and transplanting the ova can be found in papers by Dracy & Petersen (72, 73), Willett, Black, Casida, Stone & Buckner (74), Chang (75) and Kingman (76).

Referring to multiple ovulation and fertilization in cattle, a case was reported recently by

Donald (77) of a Shorthorn cow which gave birth to five live calves, one male and four females. The smallest weighed about 30 lb. and the largest 45 lb. at birth.

There is not sufficient space available here to discuss recent work on identical twin calves, but since the subject is constantly increasing in both interest and importance, reference is made to the important series of papers by Hancock of the Ruakura Animal Research Station in New Zealand who has done so much on this subject. The series begins with a paper on the organization of twin collection (78) and continues with one on the recognition of monozygotic calves (79), a subject which has also been dealt with recently by other workers both in Britain (80) and in Germany (81). Hancock's series of publications continues with papers on uniformity trials, with particular reference to milk and butterfat production (82), grazing behaviour (83), growth (84) and physiological and biochemical characteristics (85). One of the objects of this work was to determine from a comparison of the within- and between-set variance the 'twin efficiency values' or the number of randomly selected animals which one set of twins could replace without loss of statistical efficiency in experiments on milk production, milk composition, growth and behaviour. White (86) has compared the levels of some of the blood constituents in calves, and has concluded that identical twin calves have no advantage over ordinary fraternal twins in experiments involving these blood constituents, nor would these blood constituents be helpful in diagnosing zygosity in twin calves.

LACTATION

Hormones

Development of the mammary gland in laboratory animals. As explained in earlier reviews of the present series (1, 87), a theory was formulated some years ago by Turner and his associates at Missouri which postulated that there were two mammogenic hormones secreted by the anterior pituitary, one stimulating duct growth in the mammary gland, and the other stimulating growth of alveolar tissue. Since that theory was first advanced other workers have obtained at least limited mammary growth in hypophysectomized animals with oestrogen treatment alone, and it has been observed for some species that when one rudimentary teat is treated with oestrogen that particular teat develops while the neighbouring teats show little or no change, whereas if the oestrogen acted only through the pituitary all the teats would be expected to be affected. With the object of elucidating some of the intricacies involved, Trentin & Turner (88) have studied the development of the mammary gland with special reference to the interaction of pituitary and ovarian hormones. They used male rats and mice as their experimental animals. Contrary to earlier findings, the anterior pituitary factor which stimulates duct growth was found to be associated with the protein fraction and not with the fat fraction, and it appears now that the earlier observations, which were considered to prove that the duct growth mammogen and alveolar growth mammogen were different hormones, can no longer be considered valid. From this recent work Trentin & Turner conclude that mammary growth in the rat and mouse depends partly on the ability of oestrogens to stimulate the adrenal cortex, and of the adrenal cortex to secrete steroids which, like progesterone, can act along with oestrogen and together stimulate mammary alveolar development. Some of the interrelationships between the pituitary and ovarian hormones in mammary development and milk secretion have been studied also in recent years by Desclin (89) and by Lyons, Li & Johnson (90) who used female rats as their experimental animals. The ability of different oestrogens to stimulate mammary growth has been studied by Reece (91), who used more than 400 immature male rats kept on a restricted diet. He found that of nine oestrogens examined diethylstilboestrol and hexoestrol were the most active, whereas the dimethyl ethers of these substances showed least activity. There was not much difference in activity between oestradiol and its benzoate, but the dipropionate was only about half as active. Oestrone was much less effective than the same amount of oestradiol. Trentin, DeVita & Gardiner (92) have studied the effect of ovarian hormones on mammary development in dogs. Three ovariectomized dogs were given injections of 10 mg. oestradiol

benzoate daily for almost 100 days, and exhibited little or no mammary growth, whereas extensive mammary growth occurred in eight dogs given 10 mg. progesterone in addition to the oestradiol benzoate. In six dogs progesterone resulted in mammary growth, but more slowly than when the two hormones were given together.

Elliott & Turner(93) have studied the rapid proliferation of side buds which invades the adjacent subcutaneous fatty connective tissue in the mammary gland in the early stages of pregnancy. They suggested that a mucolytic enzyme or collagenase might be elaborated by the growing mammary gland cells which, by liquefying the adjacent connective tissue, might make possible the penetration of these growing buds. Their work was planned with the object of finding whether such an enzyme or 'spreading factor' is in fact produced. Assays of extracts of mammary glands from sixty-seven female pregnant rats showed that a spreading factor was present from the 3rd to the 18th day of pregnancy, the maximum concentration appearing to occur about the 12th day. The factor did not appear to be hyaluronidase. Later work by the same authors(94) showed that hormones which stimulate mammary development cause the formation or activation of the spreading factor. Maximum activation was obtained by treatment with 1 mg. oestradiol benzoate plus 5 mg. progesterone. Graded doses of either oestrogen or progesterone alone were not as effective as half the level of progesterone plus a little oestrogen. An anterior pituitary extract alone produced some mammary spreading factor, but the response was much greater when a small amount of oestrogen was also included.

The growth of the mammary glands of pregnant rhesus monkeys after removal of the pituitary on the 29th to the 156th day of pregnancy, which normally lasts 180 days, has been studied by Agate(95). Lobule length and depth, alveolar size and lumen diameter and the duct length were all found to be affected.

Development of the mammary gland in ruminants. As pointed out earlier in the present article, sterility or apparent sterility in cattle accounts for much of the wastage which occurs in dairy herds throughout the world. It is natural to find, therefore, that much work has again been done in the past two years on the 'artificial' induction of mammary development and lactation by hormone treatment. If this process could be perfected many an apparently unproductive animal could be made to produce milk and could perhaps at a later stage be brought into calf. In a most informative article on the subject by Cowie(96) an account is given of the work he and his colleagues at the National Institute for Research in Dairying are doing with the object of gaining more precise information, particularly of a quantitative nature, on the hormonal mechanisms controlling mammary growth and milk secretion, for it is only by gaining much more precise knowledge that some of the difficulties encountered when lactation is artificially induced will be overcome, and the procedure be made safe for widespread practical use. Cowie discusses experiments on the growth rate of the mammary gland of the rat from birth to maturity, and attempts to estimate the quantities of ovarian hormones necessary to maintain the normal growth of the glands. He then describes work in progress to determine the amounts of ovarian hormones required for full lobule-alveolar development in the spayed guinea-pig, and finally gives an account of hormonal induction of lactation in the goat. He points out how little is really known about the hormonal changes that occur during pregnancy in the cow and goat, and emphasizes how much could be gained if suitable methods were available for estimating hormone levels in the blood of ruminants during pregnancy. In spite of all the work that has been done, there is little definite information on the best ratio of progesterone to oestrogen for mammary development, but Cowie's results tend to suggest that within a species the ratio itself may matter much less than the absolute amounts present. For further discussion of the progesterone-oestrogen ratio and other important considerations reference should be made to a publication by Folley & Malpress(97).

Further details of their work on goats has been published very recently by Cowie, Folley, Malpress & Richardson(98). It was found that removal of one mammary gland did not affect the milk yield of the other. Single glands were therefore removed from several of the experimental animals at the peak of lactation for histological examination. The mammary glands of

treated animals had a smaller surface area of epithelium with fewer and larger alveoli than those of normally lactating goats. The calculated surface area was significantly correlated with milk yield. When different treatments were used, e.g. 0·25 and 1·0 mg. hexoestrol daily with or without 40 or 100 mg. progesterone, there were no great differences in the milk yields produced, but all were significantly lower than in normal lactation. The same authors (99) have found that when lactation was induced in goats by oestrogen treatment 3–6 mg. thyroxine daily did not appear to affect either the lactation itself or the histological structure of the mammary tissue. Reports on the induction of lactation in virgin goats have also appeared recently from India (100), Germany (101) and Japan (102). The Indian workers have studied the effect of feeding iodinated protein to goats in which lactation had been induced by stilboestrol. In some of the goats the iodinated protein appeared to augment milk secretion when the implantation and partial withdrawal of stilboestrol had successfully induced lactation. Desclin & Derivaux (103) gave eleven kids intramuscular injections of diethyl stilboestrol, and when lactation was established five were given 1–2 mg. thyroxine per day by injection and four received 15 g. iodinated casein orally per day for 4–5 weeks. A considerable increase in yield occurred in six of the animals. Szumowski & Bataille (104) and Szumowski & Charenton (105) have studied the effect of feeding iodinated casein to heifers in which lactation had been induced by oestrogen treatment. There was a tendency for the yields to be increased.

Some of the work which has been done recently on the artificial induction of lactation in cattle has been carried out by Meites, Reineke & Huffman (106) who implanted two sterile Guernsey heifers with 2 g. stilboestrol and 4 g. progesterone, and obtained rapid growth of the udder in both animals. In one of the animals, which was 4 years old, milking was begun on the 64th day and during the next 46 days, this animal produced almost 1000 lb. milk and 44 lb. butterfat. Her peak yield was 35·6 lb. of 4% fat corrected milk which compares well with first-calf Guernsey heifers. With the younger animal, which was 2 years old, only a few millilitres of milk were being obtained even by the 97th day. On the 104th day the stilboestrol pellets were removed and the milk yield began to increase. By the 10th day after removal of the pellets it was nearly 10 lb. In spite of this fair degree of success the authors point out that several problems remain to be solved before the artificial induction of lactation can be adopted in farm practice. Two of these problems are the determination of the amounts of stilboestrol and progesterone which should be used to insure optimal udder development, and the establishing of improved methods for controlling the rate of absorption of these two hormones by the blood. Sykes & Wrenn (107) have studied the effect of stilboestrol alone, stilboestrol plus progesterone, stilboestrol plus pituitary extract and stilboestrol plus both progesterone and pituitary extract on mammary development in Friesian heifers which ranged in age from 1 to 9 months. The udders which developed on treatment with both steroids and pituitary extract appeared to the authors to be more mature structurally than the udders of heifers injected with steroids only. In the treated heifers the pituitaries, thyroids and ovaries tended to be smaller and the adrenals larger than in control animals, and it was concluded from some of the results obtained that the injected hormones frequently tended to inhibit production of some of the endogenous hormones. Daily injections of 20 mg. diethylstilboestrol have been found by Schlotthauer (108) to induce lactation in a 2-year-old heifer which had persistently failed to become pregnant and in one 5-year-old cow carrying a mummified foetus. Hill (109) has referred very briefly to his experiments at Minnesota on the hormonal control of udder development in virgin heifers and multiparous cows. One of the cows responded to stilboestrol with a 295-day lactation and yielded over 500 gal. milk, but udder development could not be induced a second time in this cow. Meites, Reineke & Cairy (110) have also reported very briefly on studies they have been making to determine the optimum levels of diethylstilboestrol and progesterone for inducing udder growth and lactation in dairy cattle. They concluded that their best results were obtained by first implanting 3 g. progesterone and 100 mg. diethylstilboestrol, followed 90 days later by a second implant of 1·5 g. diethylstilboestrol. The first implant is to promote udder growth and the second to initiate lactation by stimulating prolactin secretion. After 4 months the residues of

the implants are removed and milking begun. Increase in body weights and high milk yields of up to 80 lb. per day resulted, but one of the chief problems encountered appeared to be the variable growth of fibrous connective tissue around the implanted hormone tablets; this prevented maximum hormone absorption and frequently resulted in relatively low milk production. Fuller details of these experiments will be awaited with interest. Other experiments on artificially induced lactation in cattle have been described by Peeters, Massart, Coussens & Vandeplassche (111) in Belgium, by Letard, Szumowski & Parez (112) and Szumowski & Langlois (113) in France, by Heidrich (114) in Germany, and by Borgioli (115, 116) in Italy. In the second of these last two papers, in addition to reporting the results of his experiments, Borgioli discusses the possibility of using hormone treatment not only for inducing lactation in sterile heifers, but also for increasing milk yields of beef and draft cows which often do not produce enough milk for their calves. Another Italian worker, Ferrara (117), has used udder massage with stilbene ointment and injection of stilbene compounds to induce lactation in three virgin ewes ranging in age from 6 to 15 months. The highest yield recorded was 155 ml. milk per day with 6·5% fat. Marked mammary development was noted in lambs of both sexes by Perry, Andrews & Beeson (118) in experiments in which subcutaneous implantation of stilboestrol was used to increase the growth rate. Many of the lambs yielded apparently normal milk.

Recent reviews, not so far cited, on the practical use of hormones in animal production, include one by Hammond (119) and one by Simonnet & Bars (120).

Before leaving the subject of oestrogens it will be well to draw attention to the important finding of Bennetts, Underwood & Shier (121) and Bennetts & Underwood (122) in Australia that subterranean clover possesses oestrogenic activity, and of Bartlett, Folley, Rowland, Curnow & Simpson (123) at Reading, who detected the presence of oestrogenic activity in pasture. The subject has now been further investigated by Legg, Curnow & Simpson (124), who have found considerable oestrogenic activity in ryegrass, cocksfoot grass and red clover but very little in lucerne, timothy grass and white clover. In the species which showed considerable activity it was present in the leaf, petiole, stem and inflorescence. Chloroplasts appeared to be particularly rich in the hormone. There was considerable seasonal variation, and the various parts of the plant did not show maximal concentrations simultaneously. Oestrogenic activity in spring ryegrass and clover has been found also by Dohan, Richardson, Stribley & Gyorgy (125) in Pennsylvania, but there was little activity in autumn ryegrass. The effect of feeding subterranean clover on the nipple length in castrated male guinea-pigs has been investigated by East (126) in Western Australia. The effect became obvious after 14 days, and was greatest at about 30 days. The change in nipple length was similar to that obtained in castrated animals by injecting stilboestrol.

The anterior pituitary. In 1949, Cotes, Crichton, Folley & Young (127) showed that a purified preparation of the anterior pituitary growth hormone was highly galactopoietic. In fact, it was calculated that in short-term experiments the whole of the galactopoietic activity of an anterior pituitary extract might reasonably be attributed to the growth hormone. The authors pointed out that treatment with pituitary growth hormone might induce growth, the secretion of extra milk or diabetes depending on the age, species and condition of the treated animal. In work at Minnesota, Donker & Petersen (128) have reported very briefly that injections of a growth hormone preparation into seven cows resulted in substantial increases in yield except with one animal which did not appear to be normal. In a further brief abstract (129), by the same authors, it is reported that while the growth hormone brought about an increase in yield, prolactin in two cows depressed it and a thyrotropic preparation had no effect.

Experiments with a prolactin preparation labelled with ^{131}I, but still biologically active, have been made by Sonenberg, Money, Keston, Fitzgerald & Godwin (130). When the labelled hormone was injected into immature, mature, pregnant or lactating female rats, localization of radioactivity occurred in the ovaries, adrenals, liver, spleen and kidneys, but no significant amounts accumulated in the mammary glands. The authors very wisely state that until further work is done on this method of approach, the results must be interpreted with caution.

Li(131) has continued his studies on the properties and chemistry of the lactogenic hormone of the anterior pituitary, and has now made an amino-acid analysis of the protein of his particular preparation. He used microbiological assay methods for most of the amino-acids but trytophan was determined chemically. He found that all the nitrogen of the preparation could be accounted for by his results for seventeen amino-acids and the amide nitrogen. He calculated the molecular weight of the preparation to be 33,300.

For details regarding the weights and measurements of 1282 pituitary glands from Jersey and Hereford cattle at various ages and stages reference should be made to the description of an investigation made in New Zealand by Bassett, McMeekan & Wodzicki(132). Paredis(133) has also studied the weights and dimensions of the pituitary glands of cattle, particularly in relation to the oestrous cycle and stage of pregnancy. Neither appeared to affect the size of the anterior lobe.

The adrenal hormones

Relatively few publications have appeared in the past two years on the part played by adrenal hormones in lactation. In a brief abstract, Smith(134) has outlined experiments in which adrenalectomized rats, given a solution containing salt and glucose, had normal pregnancies followed by normal parturition but failed to lactate. In comparable animals given 0·1 or 0·5 mg. deoxycorticosterone acetate and ordinary tap water lactation was poor, but when the amount of hormone was increased to 1 mg. per day it was much improved and with 2 mg. it became normal. Cowie(135) has reported that when cortisone was implanted in adrenalectomized lactating rats lactation was partially restored, and that when both cortisone and deoxycorticosterone acetate were implanted complete restoration of lactation appeared to result.

Balmain & Folley(136) have studied the effects of prolactin and cortisone on the metabolism of rat mammary tissue *in vitro*. They found that when the mammary tissue was taken from a 20-day pregnant rat cortisone stimulated the respiratory metabolism, but in the same tissue prolactin did not have an effect. When, however, the mammary tissue was taken *post partum* cortisone no longer affected the respiratory metabolism, whereas now the respiratory metabolism was increased by prolactin. The authors suggest the possibility that full saturation with cortical hormones is necessary for the tissue to respond to prolactin. For a valuable review of present knowledge on the interaction of pituitary and adrenal hormones in the initiation and maintenance of lactation, which includes references to earlier work, the reader should consult a recent article by Folley(137).

Thyroxine and iodinated protein. During the past two years there have been few publications devoted solely to fundamental work on the part played by the thyroid gland in milk secretion. Most attention has been given to practical field studies on the effect of treatment with thyroxine or iodinated protein on milk production and on the health of cows. In the previous review of the present series(1) detailed reference was made to an excellent article on this subject by Blaxter, Reineke, Crampton & Petersen(138) and the main conclusions arrived at by these workers were listed. A shorter article has since been published by Blaxter(139), in which the main findings of the experiments carried out in the past decade have been summarized and a helpful account given of the risks which would be involved in adopting thyroxine or iodinated protein treatment in farm practice. The same author has also contributed a chapter on the subject to the most recent volume of *Vitamins and Hormones*(140). Since the earlier of these reviews were published, papers have appeared by workers from various countries in which descriptions are given of the results of treating cows with thyroid-active materials. Usuelli & Piana(141) have recorded the results of several years' investigations in Italy made chiefly with Friesian cows. They claim average increases of about 9% in milk yield and 21% in butterfat, the more vigorous responses being obtained from cows with initially higher yields. High environmental temperatures were found to reduce the response. In Urbana, Gardner & Millen(142) have studied the effect of feeding iodinated protein to cows during the summer months when atmospheric temperatures were high. A 15-week double reversal study was made with four cows, each of different breed, and a continuous trial with eight Holstein cows. In the latter trial,

where milk production decreased 8% in the controls over an 8-week period, the cows receiving 1·33 and 2·0 g. iodinated protein per 100 lb. body weight daily showed increases of about 12 and 22% respectively. The efficiency with which total digestible nutrients were utilized in treated animals was calculated to be equivalent to about 70% of what it was in controls. Body weights of treated animals declined rapidly in the experimental periods; respiration rates, pulse rates and body temperatures increased. When thyroprotein feeding ceased milk yields declined rapidly and body weights increased. These results are typical of those which have been obtained in many parts of the world in experiments on the value of iodinated protein for increased milk production. Leech (143) has concluded from experiments involving fifty-nine cows in which treatment of the experimental animals began 3 months after they calved, and continued for 28 weeks, that it would be bad practice to continue iodinated protein feeding for periods of more than 8 weeks, for he found that after 10 weeks the increased production either ceased to exist or was greatly diminished. Experiments extending over two or more successive lactations have been described by Thomas & Moore (144) and Swanson (145). Swanson fed 15 g. thyroprotein per day to five cows in two successive lactations for about 200 days, beginning about 130 days from parturition. The increased milk-yield response lasted about 70 days in both lactations, but the overall benefit was small. No harmful effect was observed as a result of this moderate dosage, but histological examination revealed that the thyroid gland of cows fed the iodinated protein for 150 days was practically inactive. This is taken to indicate that the administration of thyroid-active substances diminished the secretion of the thyrotrophic hormone by the pituitary. Several cows were treated with iodinated casein over three consecutive years by Schuurmans (146) who found no injurious effects. He concluded, however, that the advantages gained were not sufficient to justify the risks which would be involved in the application of iodinated protein feeding on commercial farms.

A few years ago Blaxter (147, 148) showed that when sheep were given iodinated casein the metabolic rate was linearly related to the dosage expressed in mg. per kg. $W^{0·73}$, and that the endogenous nitrogen excretion also increased linearly with dosage. Following that work, experiments have now been carried out by Mukherjee & Mitchell (149) in which a study has been made of the effect of feeding iodinated casein on the metabolic rate and endogenous urinary nitrogen excretion of two Holstein bull calves weighing initially 270–340 lb. With the dose given the metabolic rate increased by 31 and 35%, and the endogenous urinary nitrogen by 19 and 16% per sq.m. of body surface. Thus both the basal expenditure of energy and the endogenous catabolism of nitrogen were enhanced. From their results the authors calculate that the cost of the extra milk produced when iodinated protein is given to cows, when regarded in terms of the total digestible nutrients required, might well be 100% greater than the normal cost, and that the cost in terms of digestible crude protein might be 50% above normal. The increase which occurs in the metabolism of cows treated with iodinated casein has been studied by Moustgaard & Thorbeck (150), whose work was referred to in the previous review (1), and who have now given a further description of some of their main findings. The increases they found in the metabolic rate of cows (151) were similar to those observed for calves in the work already cited by Mukherjee & Mitchell (149).

Other papers on iodinated protein or thyroxine, which have appeared in the past two years and which may be mentioned here, include descriptions of experiments on the elimination of iodinated compounds or iodides labelled with radioactive iodine in the milk of sheep (152) and rabbits (153).

The effect of feeding iodinated casein on semen production in bulls has been studied by Sørensen & Hansen (154). It appeared to have a deleterious effect on the quality of the semen and is not recommended.

A long-term investigation is being made by Owen and his colleagues (155–161) on the effect of thyroxine and thiouracil on the composition of the milk of cows and goats, with particular reference to some of the substances such as enzymes, vitamins and minerals which are present in milk in only small amounts but which are so important nutritionally. They have also studied

the effect of these two substances on the metabolism of carotene and vitamin A in lactating goats. They have found that when thyroxine treatment decreases the phosphatase content of milk it increases markedly the synthesis by the mammary gland of compounds containing phosphoric esters; at the same time the content of inorganic phosphate in the milk is reduced. Other effects of thyroxine are an increase in the cocarboxylase content and in the amount of protein-bound thiamine. There is also a reduction in ascorbic acid, an increase in the amount of carotene passing into the milk and an increase in the amount of carotene transformed by the animal into vitamin A. These last effects are associated with a marked increase in the digestibility of carotene in cows treated with thyroxine. For all the changes observed to be brought about by thyroxine, thiouracil had the reverse effect.

Campbell, Hollard & Flux(162), in New Zealand, have made a detailed investigation of the weights of thyroid glands from several classes of cattle from different districts. There was no significant difference between the mean thyroid weights of males and females.

The effect of iodinated protein on human lactation has been studied by Roche, Giraud, Lelong, Liardet & Coignet(163). Doses varying from 40 to 400 mg. per day were given orally to thirty-one women at various stages of lactation. Responses in yield are said to have varied from a decrease of 26% to an increase of 15%, and change in fat production from −33% to +200%. The authors suggest that in some cases daily administration of 100 mg. iodinated casein for fortnightly periods, alternating with a week without treatment, might be of value.

Milk ejection. It was in 1941 that Ely & Petersen(164) put forward their theory of milk ejection which is now so well known. These authors suggested that many sources of afferent stimuli such as handling the teats, rattling milk buckets and washing the udders with warm water stimulate the posterior pituitary through the central nervous system to secrete a hormone (oxytocin) which increases the glandular pressure and squeezes the milk from the alveoli and smaller ducts, whereas afferent stimuli of a different nature such as fright cause the secretion of adrenalin which prevents the ejection of milk. The term 'milk ejection' is strongly recommended by Cowie, Folley, Cross, Harris, Jacobsohn & Richardson(165) in place of terms such as 'let-down'. It is hoped that this recommendation will be universally adopted.

The subject was reviewed critically in 1950 by Macaulay(166), who expressed the opinion that 'it has certainly not been proved, as Petersen and his colleagues have claimed, that the neural lobe plays an endocrine role in milk ejection'. She admitted that some substance had been detected in the blood of stimulated animals which could affect a more than normally rapid ejection of milk in the isolated udder, and that extracts of the posterior pituitary gland possess this property, but she held the view that more information was required on motor innervation of the udder, on blood flow in the lactating gland, on the effect of neural lobe destruction in normal lactation, and on the identification of a neural lobe activity in the blood of animals stimulated to eject milk. Since Macaulay published her review, further evidence has become available on various aspects of the subject.

Within the past three years Richardson(167) has made an intensive histological study of the nature and distribution of the myoepithelium and of smooth muscle in the lactating gland of the goat. For this purpose he evolved a valuable method of staining myoepithelium which depends on silver impregnation, and which has now been used also by Linzell(168) with other species. Both authors conclude that the myoepithelium tissue is ideally arranged around the alveoli and along the ducts for squeezing milk out of the acini and small ducts into the larger ducts and cisterns. There seems now to be little doubt that the myoepithelial cells act as contractile elements and have an active part to play in the ejection of milk.

In recent years Whittlestone, in New Zealand, has made a study of the milk-ejection process using the lactating sow as his experimental animal. The work was made possible by the introduction of a milking machine for use with sows(169), animals which, as Whittlestone(170) explains, have many advantages for this type of work. Extracts of the posterior lobe of the pituitary can be fractionated into two components, one predominantly oxytocic with less than 5% of pressor contaminant, and the other a pressor fraction with less than 5% oxytocic contamination.

Ely & Petersen(164) demonstrated that both fractions stimulate milk ejection, and concluded that the principle concerned was the oxytocic principle, the activity of the pressor fraction being ascribed to contamination. Whittlestone(170) tested the activity of a number of posterior pituitary preparations against a standard oxytocin preparation about 1 hr. after normal suckling, and came to the conclusion that purified vasopressin has milk-ejecting activity roughly equal to one-fifth of that of the equivalent number of oxytocic units, but he stresses that further work is required with pressor material which is entirely free from possible active contaminants. In another paper(171) Whittlestone concludes from his work with sows that the milk-ejection hormone not only causes a contraction of the myoepithelium around the alveoli so increasing the pressure within them, but also causes an opening of the small ducts due to the tightening of the longitudinal myoepithelial structures which surrounds these ducts. This results in a rapid flow of milk into the large ducts and cisterns. Some of the factors, such as age, which may affect the amount of milk-ejection hormone present in the posterior lobe and the frequency of its discharge have been studied recently by Whittlestone, Bassett & Turner(172, 173). Other accounts of Whittlestone's work have been given recently in two New Zealand publications(174, 175).

Other work on the sow has been described by Braude & Mitchell(176), who injected doses of oxytocin consisting of 1, 3, 5 and 10 units. It was clear from the results that the amount of milk released by a sow is related to the concentration of oxytocin in its blood. For the four increasing doses the average milk yields were 41, 78, 82 and 104 ml., and the times for which the milk flowed were 80, 131, 135 and 179 sec. The time which elapsed between the end of an injection and the beginning of the milk flow diminished from 11·6 to 9·9 sec. with increasing dose.

Other work on the subject in Britain has been done in the past few years by Cross & Harris(177, 178, 179) and Cross(180). In a detailed report of a lecture given by Harris(181) the number and nature of the hormones secreted by the posterior pituitary are discussed, and a description is given of experiments made with rabbits in which a study has been made of the secretory activity of the gland when stimulated in several different ways. Direct electrical stimulation of the hypothalmic region caused the liberation of the oxytocic hormone as judged by the fact that ejection of milk occurred similar to that produced by injections of oxytocin, and it was found that lesions in the region of the supraoptico-hypophysial tract prevented the initiation of the milk-ejection reflex by the suckling young. Harris & Jacobsohn(182) have made grafts of anterior pituitary tissue from rats into various positions around the brain of hypophysectomized adult female rats. When the grafts were situated under the median eminence of the tuber cinereum normal reproduction and milk secretion occurred, but milk could be obtained by the young only when oxytocin was injected.

In Belgium, various aspects of the subject have been investigated by Peeters and his associates. In one section of their work(183) they used the isolated perfused udder of the cow to study the effects of nerve stimulation and of various pharmacological substances. Electrical stimulation of the inguinal nerve caused a vasoconstriction and a slight decrease in cistern pressure similar to that obtained with adrenalin. They concluded from their experiments that the antonomic nerve supply to the udder is probably sympathetic in origin. In another section of the work(184) they studied cistern pressure relationships in the intact and isolated bovine udder. The injection of 3 units of pitocin caused an increase equivalent to 24 cm. of water in the cistern pressure whether the initial milk pressure was low or high. Perfusion with blood from a freshly milked cow also caused a slight increase, whereas adrenalin brought about a decrease equivalent to 3–10 cm. of water in the cistern pressure. In experiments on the effect of various pharmacologically active substances on milk ejection in the isolated udder Peeters, Sierens & Silver(185, 186) found that acetylcholine caused increased blood flow and partial ejection, and that this action was inhibited by atropine. A table summarizing the effects of various pharmacologically active substances on milk ejection and blood flow in the perfused mammary gland has been given by Silver(187).

In Stockholm, milk ejection has been investigated by Andersson(188, 189) using sheep and goats. He found that electrical stimulation in the area of the supraoptic nuclei resulted in ejection of

milk which must have been brought about by hormone action since it was unaffected by sacral anaesthesia or by unilateral denervation of the udder. Blood taken from an animal so stimulated caused increased flow of milk in another animal. Like other workers, already referred to, he found that the activity of pitressin was greater than could be explained by its oxytocin content. From the same laboratory Andersson & Larsson (190) have confirmed that when the posterior pituitary is stimulated both oxytocic and antidiuretic hormones are secreted simultaneously; diuresis in rabbits was inhibited by injecting 6 ml. of cows' urine taken 30 min. after milking, whereas urine taken before milking did not have that effect.

Work on milk ejection has been reported also from Italy by Piana (191) and from Russia by Grachev (192).

The knowledge that has accumulated on milk ejection in the past decade has led to much study of the actual technique of milking itself, for at first it appeared probable that to get the highest yield at a milking and to get the milking process completed as rapidly as possible, milking should be done while the effect of oxytocin secretion is at its height. This was discussed in the previous review (1) in which reference was made to the series of papers being published by Dodd and Foot on milking technique. That series of papers has been continued in the past two years. The earlier experiments were of a short-term nature and the cows used had previously been accustomed to other routines. In some of the recent work (193), therefore, thirty-eight first-calf heifers were divided into two equal groups, and a study made of the effect on milking rate of subjecting the cows to a fixed milking time of either 4 or 8 min. at every milking throughout a complete lactation. No significant changes in milking rate were brought about by restricting the milking period to the shorter time, and although the teat-cups were often removed from the cows in the 4 min. group when milking was still in full flow and were left on longer than necessary in the 8 min. group, the milk yield in the 4 min. group was significantly less than for the other group only in the first 6 weeks of lactation and the fat yield only in the first 21 weeks. For the complete lactation there were no significant differences in the total yield of milk and milk fat, nor was there any significant difference in persistency. Popular descriptions of this work, and its application in practice, have been given recently by Dodd (194) and Foot (195).

Beck, Fryer & Roark (196), Beck & Claydon (197), and Beck, Fryer & Atkeson (198) have described experiments at Kansas Agricultural Experimental Station in which they have adapted a continuous-feed kymograph to obtain continuous records of the rate of milk flow. They have used this apparatus to study milk-flow rates under different milking techniques, and for the same technique applied to animals of different breeds. They concluded that the speed at which milk can be withdrawn is an inherited characteristic, since they found significant differences not only between breeds but also between six families within a breed. The rate was greatest in Holsteins. The results indicated that attempts to train slow milkers to respond at a faster rate would not be likely to meet with marked success.

Since space in this article is strictly limited and since the object is to discuss physiological aspects of the problem rather than milking techniques, it is impossible to deal adequately here with other papers which have appeared on the subject in the last two or three years. It will only be possible to mention that work on various aspects of the general problem have been studied recently by Swanson & Hinton (199) at Knoxville, Knoop & Monroe (200) at Ohio, Kirsch & Parau (201) at Kiel, and Evseev (202) in Russia.

Further work on the milk-ejection reflex in human lactation has been continued by Newton & Newton (203).

BIOCHEMICAL ASPECTS

Three techniques which have been used greatly in recent years in attempts to elucidate some of the processes involved in milk secretion are the perfusion of the excised udder, the incubation of mammary tissue slices, and work with isotopes of carbon, hydrogen, phosphorus and other elements.

The perfusion technique. The perfusion technique has been reviewed recently by Silver (204, 187),

who has given a brief description of the perfusion methods used by different workers and an appraisal of the main physiological and biochemical studies which have been made in recent years with the aid of the perfusion technique. She points out that, although valuable information has undoubtedly been obtained by this procedure, the methods employed are still far from precise and mentions tissue damage, rate of blood flow through the perfused udder, depletion of reserves and the balance of blood metabolites as some of the factors which are difficult to control and which tend at present to render perfusion experiments less well controlled than some other *in vitro* techniques.

Since the last review of the present series was published (1), Peeters & Massart (205), in Belgium, have given a detailed description of their method for perfusing the two halves of the bovine udder separately so that one half can be used as a control; the apparatus they use is shown in diagrammatic form. Usually the experiment does not take longer than 2 hr. and the blood of the animal which provided the udder is used for the perfusion. Peeters and his colleagues found this method of value in studies on the physiology of the cistern (206) and on the innervation of the mammary gland (207), but at first its value for biochemical studies was limited owing to the fact that only a limited quantity of blood could be used. Its authors are, however, developing the method further. Some of the factors which affect the flow of blood through perfused mammary glands have been very fully discussed recently by Hebb & Linzell (208), who have described the perfusion of cat, dog and goat mammary glands by a method in which the lungs are included in the circuit. By this technique it was possible to obtain greatly improved rates of blood flow. This was believed to be due to the removal in the lungs of the vasotins present in shed blood, and also to a reduction in their formation when a more rapid flow through the apparatus is maintained.

Protein formation. Bouckaert, Oyaert & Peeters (209) have used their technique to study the uptake of amino-acids by the mammary gland. The two halves of the udder were perfused separately for 90–120 min. with 9 l. of oxygenated heparinized blood at 38° C. Ten essential amino-acids were estimated microbiologically in the plasma. The authors found that the decreases which occurred in the amino-acids of the plasma as it passed through the gland were 'more or less proportional' to their concentration in the milk proteins, the greatest decreases occurring in isoleucine, leucine, lysine and valine. In the United States, Barry (210) has used Petersen's perfusion technique (211) to study the uptake by the excised lactating mammary gland of the cow of lysine and tyrosine labelled with ^{14}C. He also injected these substances intravenously into a lactating goat and measured the specific activities in various components of the blood and milk. He concluded that all the lysine and tyrosine for the casein of the milk was provided by the free amino-acids of the plasma and that plasma protein was not used for casein synthesis to any significant extent. He also used ^{32}P in the same experiments, and concluded that plasma inorganic phosphorus provides all the casein phosphorus. On the average in the goat, lysine, tyrosine and phosphorus took about 2 hr. to pass from the blood stream to a state in the mammary gland in which they could be obtained as casein in the milk. The fact that free amino-acids are removed from the plasma by the lactating mammary gland and converted to milk protein has also been confirmed recently in rabbits by Campbell & Work (212), using valine and lysine labelled with ^{14}C. From further experiments by the same authors (213), in which labelled glycine, lysine, valine and methionine were injected into lactating goats and rabbits and in which a rabbit mammary gland was perfused with blood containing labelled plasma proteins, it was concluded that milk proteins are derived mainly from the free amino-acids of the plasma, but the possibility that a very small proportion of the protein was formed from peptides derived from plasma protein was not excluded. When $NaHCO_3$ labelled with ^{14}C was injected intravenously into lactating cows it was concluded by Kleiber, Smith & Black (214) that in their particular experiments 4% of the carbon of casein originated from carbonate. On hydrolysing the casein from the milk (215), and separating the amino-acids chromatographically, it was found that all the amino-acids contained some labelled carbon; by far the greatest amounts were in the non-essential amino-acids, but measurable amounts were also detected in

the essential amino-acids. In the essential, but not in the non-essential acids, the labelled carbon was all in the carbonyl radicals suggesting that CO_2 fixation in the molecules of the essential amino-acids results from a reversible decarboxylation process. The same workers, in further studies with labelled bicarbonate, acetate, propionate and butyrate (216), concluded that in synthesizing amino-acids from precursors in the blood, cows behave similarly to rats and dogs in that only the amino-acids known to be non-essential for rats and dogs are formed. If, therefore, the so-called essential amino-acids are not essential for cows it must be because they are synthesized in the rumen. They concluded that acetate was the major precursor for transferring carbon to the non-essential amino-acids, and when acetate labelled with ^{14}C in the carboxyl group was given 1% of it appeared in casein, whereas when the acetate was labelled in the methyl group the corresponding figure was 2% (217). This work does not necessarily mean that carbonate or acetate are used to any extent as precursors of milk protein by the mammary gland, because since the experiments were done with intact animals, the main site in which the radioactive elements would be introduced into the amino-acid molecules would almost certainly be the liver.

Lactose formation. In the experiments just referred to by Kleiber, Smith, Black, Brown & Tolbert (217), when cows were injected with acetate labelled in the methyl or carboxyl groups, the same proportions of the injected ^{14}C appeared in the lactose as in the protein of the milk. When labelled $NaHCO_3$ was given again an appreciable amount of ^{14}C appeared in the lactose (214). Cowie *et al.* (218) have perfused the two separate halves of a lactating cow udder, one with $CH_3{}^{14}COONa$ and the other with $NaH^{14}CO_3$. Lactose from the half which received acetate was appreciably radioactive, whereas lactose from the bicarbonate half was not. It is pointed out that the results of Kleiber *et al.* (214) may have been due to $^{14}CO_2$ being incorporated in the glycogen of the liver which would ultimately provide ^{14}C glucose for lactose synthesis. It is not suggested by any of this work that acetate or carbonate is an important precursor of lactose in the mammary gland itself. In fact, in work by French, Popjak & Malpress (219), in which ^{14}C starch was given to lactating rabbits by stomach tube and the lactose isolated and hydrolysed to glucose and galactose, the authors concluded that both parts of the lactose molecules came from the glucose of the plasma and that there was no necessity to postulate any other precursor of lactose. In this connexion it may be pointed out that Topper & Stetton (220) have concluded, from experiments with rats, that galactose-1-^{14}C can readily be changed to glucose-1-^{14}C *in vivo* by a direct epimerization at the fourth carbon atom without the molecule being broken up to give smaller units. This change may well be reversible in the lactating mammary gland. Reithel, Horowitz, Davidson & Kittinger (221), in Oregon, have studied lactose formation by incubating homogenates of guinea-pig mammary gland with various possible precursors. Again the synthesis of lactose from glucose was demonstrated by using ^{14}C glucose. Several sugar phosphates were tested as possible precursors, but most of them were inactive and none was more effectively utilized than glucose itself.

Fat formation. As a result of the outstanding work of Folley, Popjak & French referred to in the previous review (1), knowledge of the processes involved in the formation of milk fat has made great advances. That important work has been continued and extended with great success in the past two years. In 1950, Folley & French (222) published details of experiments in which mammary tissue slices from cows, goats, sheep, rabbits and rats were incubated with acetate and glucose, either together or separately, and the respiratory quotient and acetate utilization studied. Mammary tissue from all three ruminants utilized acetate to a marked extent with R.Q. greater than 1, whereas with glucose there was little activity and the R.Q. was less than 1. In the rat and rabbit mammary tissue the addition to the substrate of glucose but not acetate resulted in R.Q.'s greater than 1. When the two were added together glucose was found to stimulate acetate utilization in the tissue of the sheep, rabbit and rat. The results pointed conclusively to the fact that acetate was utilized by the mammary glands of ruminants to form at least part of the fat secreted in the milk. Following this work Popjak, French & Folley (223) injected $CH_3{}^{14}COONa$ into the jugular vein of a lactating goat and studied the respired CO_2 at

intervals for 6 hr. after injection, and the blood and milk at intervals up to 48 hr. when the goat was killed. The acetate was rapidly oxidized, and in the first 6 hr. 80% of the ^{14}C appeared as respired CO_2, the estimated half life of the acetate in the body being less than 50 min. Of the remaining 20% half appeared in the milk fatty acids in the first 6 hr., which indicated that acetate was rapidly used for milk-fat synthesis. Since the plasma fatty acids contained much less ^{14}C than those of the milk, it was concluded that the plasma fatty acids were not the main source of the milk fatty acids. For a similar reason it was clear that milk cholesterol was synthesized in the mammary gland. Since the lower fatty acids of the milk fat had a higher specific activity than the long-chain acids it was concluded that the lower fatty acids were almost certainly formed from acetate, and that they were probably intermediate in the formation of long-chain acids. This, of course, did not exclude the probability indicated by arterio-venous measurements in earlier years (1) that at least a portion of the higher fatty acids of milk fat come directly from the blood, but it did indicate that the lower fatty acids are a product of synthesis from small molecules rather than of oxidation of higher fatty acids.

The lower fatty acids from the milk collected up to 12 hr. and between 12 and 48 hr. after injection of the labelled acetate were investigated further by Popjak, French, Hunter & Martin (224), and a study made of the relative amounts of ^{14}C in the various positions of the fatty acid chains. For this purpose a method of fatty acid degradation introduced by Hunter & Popjak (225) was used. The results indicated that about 40% of the butyric acid was formed in the mammary gland by the methyl group of one acetate molecule combining with the carboxyl group of another, the remaining 60% coming from some non-isotopic C_4 compound which might be β-hydroxybutyric acid. The other acids appeared to be formed by a stepwise elongation of a shorter chain by the addition of a two-carbon unit at the carboxyl group of the shorter acid. These various findings have been confirmed by the perfusion experiments of Cowie *et al.* (218) to which reference has already been made. Petersen and his colleagues (Mix, Petersen & Struss (226)) have also perfused the isolated bovine gland with $CH_3{}^{14}COONa$. In the first 3 hr. radioactivity was detected in ether extracts and glycogen from the gland but not in the milk, but when perfusion was continued for 5–6 hr. radioactivity was observed in the volatile and non-volatile fatty acids and also in the cholesterol, casein and several other constituents of the milk. Labelled CO_2 was also found in the venous blood. There is, therefore, ample evidence that acetic acid is utilized by the mammary gland of ruminants for fat synthesis, but perfusion experiments reported recently by Peeters & Massart (227) and Peeters, Massart & Cazier (228) indicate that propionic and butyric acids are not utilized in this way.

The metabolism of pyruvate has been investigated by Terner (229) in mammary tissue slices of the rat, rabbit, sheep and goat. When 2, 4-dinitrophenol was present the oxidation of pyruvate was increased but the pyruvic acid itself did not disappear any faster, indicating that some synthetic reaction was being inhibited. It was concluded that while pyruvate is oxidized in the lactating mammary gland in the Krebs cycle (230), it can also be used for synthetic reactions, and that it is probably of importance in fat synthesis where it may supply both metabolic energy and the two carbon units required for the formation of fatty acids.

In a paper published in 1950, Achaya & Hilditch (231) put forward evidence in support of their view that breakdown of higher fatty acids to lower acids was the most likely explanation for the existence of the lower fatty acids in milk fat. Their conclusion was based on a very detailed and comprehensive study of the structure of the glycerides of cow and buffalo milk fats. They emphasized that work on the precursors of milk fat had been concentrated on possible non-fatty precursors, and that experiments should be made with higher fatty acids to determine whether or not lower fatty acids can arise also from them. This paper by Achaya & Hilditch was published before details of the very recent work of Folley, Popjak and their colleagues became available, and although that work puts beyond dispute the fact that a large proportion of the lower acids arise by synthesis from acetic acid, it would be valuable, if the difficulties of technique could be overcome, to determine whether a fatty acid like oleic acid can be oxidized by lactating mammary tissue to give lower acids. It would appear that the possibility that a portion of the

lower acids could arise in this way is not entirely ruled out, though certainly it now seems much less likely than it did a few years ago.

A detailed study is being made by Folley and his colleagues (232, 233, 234, 235, 236) of the effect of insulin on fat synthesis in tissue slices of lactating mammary glands. Insulin had a marked stimulating effect on fat synthesis in tissue slices of rat lactating mammary gland when glucose plus acetate formed the substrate, but when the slices were taken at the end of pregnancy or 2 days after weaning there was no response to insulin. Rabbit mammary tissue behaved similarly, but with ewe mammary slices insulin had no effect whether the substrate was acetate alone or glucose plus acetate. Glycerol had a similar effect to that of insulin in rat mammary tissue and the effects of glycerol and insulin were not additive. In ewe tissue glycerol was active with acetate alone but not when glucose was also present. It was suggested, therefore, that when insulin has a stimulating effect it may be due, at any rate in part, to increased glycerol formation from glucose, and possibly also to the fact that in non-ruminants it may result in an increased utilization of glucose as a source of energy for fat synthesis. In some of the later work (235) the effect of glucose and glycerol on the uptake of acetate by mammary tissue from rats and sheep has been confirmed by experiments involving labelled acetate. The same workers have now shown (236) that cortisone decreases the rate of fat synthesis from glucose plus acetate in actively lactating rat tissue and inhibits the stimulatory effect of insulin. Mammary tissue from pregnant rats was found to be sensitive to cortisone but not to insulin. The authors are continuing and extending this work. Hills & Stadie (237) have concluded that tissue slices of rat mammary glands combine rapidly with insulin in such a way that it cannot readily be removed, for even after prolonged washing of the tissue, the insulin still caused an increase in fat synthesis in the presence of glucose and acetate. In the same paper they state that insulin had no effect on the synthesis of lactose from glucose.

By a study of the specific activities of lactose and glycerol from the milk and milk fats obtained in the experiment by Popjak *et al.* (223), already referred to, and by studying the distribution of radioactivity in the glycerol molecule, Popjak, Glascock & Folley (238) have demonstrated that glucose is the carbohydrate precursor of glycerol in the udder.

For valuable reviews, which deal mainly with fat metabolism in the mammary gland and which can therefore enter further into the literature than is possible in a more general article such as this, the reader should consult publications by Folley (239), Popjak (240, 241, 242), and French (243).

General. In a lengthy and detailed paper, McClymont (244) has described some of his important experiments on volatile fatty acid metabolism in ruminants. The levels of acetic acid in the rumen and in the arterial blood were found to be similar to each other in cattle and sheep, and there was a close relationship between changes in the rumen levels and those of the arterial blood. Under the particular conditions of his experiments McClymont found that the acetic acid content of arterial blood reached a maximum of 8–14 mg./100 ml. 2–5 hr. after feeding and fell to 2–6 mg./100 ml. 16 hr. after feeding. After a fast of 72 hr. values were as low as 1·5 mg./100 ml. Arteriovenous differences as the blood passed through the mammary gland were concluded to be dependent on the arterial level. They were of the order of 2–6 mg./100 ml. or 40–80% of the arterial level in the fed animal. From some of the results recorded in the paper the author concluded that while acetic acid is utilized for fat synthesis and for oxidation in the mammary gland, the proportion of lower fatty acids in milk fat does not depend on the amount of acetic acid taken up. It would obviously be of value to have this point studied in greater detail. Craine & Hansen (245) have found that as age advances and the rumen develops in very young goats blood glucose decreases markedly and the content of lower fatty acids in the blood increases. The blood of young goats taken 5 hr. after feeding contained 3–4 mg. total volatile acids/100 ml. and of adult goats 4–6 mg./100 ml., of which about 75% was acetic acid. The effect of feeding supplements of calcium acetate and butyrate to cows has been studied by Zelter (246, 247), who claims that in some circumstances the supplements increased the milk yield and fat production compared with control animals, and suggests that his results may supply evidence for the direct utilization of these substances for the production of fat.

The effect of the quantity and type of roughage fed to cows on the fat content of milk has been discussed by McClymont (248). A reduction in roughage in the ration was accompanied by a decrease in fat percentage, but not in the level of non-fatty solids.

Unusual fatty acids in milk fat. It has generally been assumed that milk fat contains all the even-numbered straight-chain saturated fatty acids from C_4 to C_{20}, and that it does not contain odd-numbered, branch-chain fatty acids or more than traces of a fatty acid with 18 carbon atoms and three double bonds. Recently, however, Hansen & Shorland (249) have shown the presence of two isomeric branch-chain fatty acids containing 17 carbon atoms and tentatively suggest that they are the *iso* and *anteiso* isomers of heptadecanoic acid. This is the first time that naturally occurring *iso*-fatty acids with an odd number of carbon atoms have been reported. The same authors (250) have also isolated from butterfat a C_{20} saturated acid with either three or four methyl groups; though saturated it melts below $-70°$. Branch-chain acids appeared to account for perhaps a little under 1% of the total fatty acids. It seems possible that they might be formed by a synthetic process in which two carbon atom units are added on in a stepwise elongation procedure to the carbon-chain portion of a deaminated branch-chain amino-acid. Lately, also, Shorland (251) has shown that in a sample of New Zealand butterfat there was about 3% of an octadecatrienoic acid which was shown by its hexabromide to be linolenic acid. Schaffer & Holm (252) have concluded that samples of butterfat which they studied in the United States contained just a little over 1% of an octadecatrienoic acid.

Various elements. The use of radioactive elements to study the secretion of phosphorus, calcium, copper and other elements has increased steadily during the past few years. Simonnet and his colleagues have used a technique in which $Na_2H^{32}PO_4$ was injected subcutaneously into a lactating goat (253) and also into lactating rats (254). Labelled phosphorus appeared in the milk of the goat 2–4 hr. after injection, the maximum being reached about the 24th hr. The amount decreased thereafter during the following 14 days. About 23% of the ^{32}P secreted in the milk was contained in the casein, and none of it occurred in the lipids of the milk. In further work by Bars, Simonnet & Sternberg (255) $Na_2H^{32}PO_4$ was injected into a lactating bitch which was killed 5 days later and the mammary glands dissected. About 31% of the total labelled phosphorus in the gland was in the protein, 50% in the total acid-soluble fraction, 10% in the ribonucleic acid fraction, 4% in the deoxyribonucleic fraction and 4% in the lipids. Saarinen, Comar, Marshall & Davis (256), who gave labelled sodium phosphate orally to a cow in mid-lactation, found that the blood showed marked activity within an hour. Two maximum values for the activity were observed. One occurred 5 hr. after the labelled phosphate was given and the other at about 30 hr. The first was due mainly to acid-soluble phosphates and the second to both acid-soluble and phospholipin fractions. Maximum activity in the phosphorus of the milk and casein was reached in 12–24 hr. The authors concluded that both the acid-soluble phosphorus and the casein phosphorus of milk arise mainly from the acid-soluble phosphorus of the blood and not from its phospholipin phosphorus. A method of making radiographs of mammary tissue after injecting the rabbits with radioactive ^{32}P derivatives has been described by Lundahl, Meites & Wolterink (257). By its means the distribution of ^{32}P in the growing and non-growing gland can be studied.

Garton & Popjak (258) have published a preliminary abstract of work which they are doing on the nature and turnover of mammary gland phospholipins for which they are using phospholipins labelled with ^{32}P.

Studies have been made in recent years of the secretion in milk of ^{45}Ca in goats (259, 260) and of ^{89}Sr, ^{90}Sr, and ^{90}Y in rabbits (261). The amount of calcium absorbed from the alimentary tract appeared to be closely related to the requirements of calcium for lactation (260). Experiments with Na_2SO_4 containing ^{35}S have shown that in lactating rats sulphate is secreted in the milk and utilized for tissue synthesis in the suckling young (262). Doses of ^{65}Zn and ^{64}Cu have been given orally to cows. Maximum activity occurred in the milk 35 hr. after administration for zinc (263) and 40 hr. after for copper (264).

Enzymes. Attention has already been drawn to a paper by Chanda & Owen (155), in which they

describe some of the effects of thyroxine and thiouracil treatment on the composition of cows' milk. They found that thyroxine resulted in a decreased secretion of alkaline phosphatase in the milk, whereas thiouracil caused an increase. The phosphatase concentration was negatively correlated with that of ester phosphorus, and with lipid phosphorus, and positively correlated with inorganic phosphate. A study of the alkaline phosphatase content in the mammary gland of the rat has been made by Goto & Ugami (265). They found that the amount present was minimal in the non-pregnant non-lactating animal, that it attained a maximum just after parturition and decreased slowly during lactation. An acid phosphatase of cows' milk capable of hydrolysing phosphoric esters at pH 4·0 has been studied in detail recently by Mullen (266). The enzyme concentration in the milk increased from a low initial value until the 5th or 6th day of lactation; it then decreased steadily until about the 100th day and remained roughly constant until near the end of lactation when it began to rise again. All four quarters of normal cows gave milk containing similar concentrations. In the milk of infected quarters the amount appeared to be enhanced, but this was probably due to *Streptococcus agalactiae* which can show a similar activity. Mullen (267) has also studied the acid phosphatase present in the mammary tissue of the cow (optimum pH 5·5–5·8) and the rat (optimum pH 6·0). The acid phosphatase of cow mammary tissue was inhibited by a factor in raw milk which was destroyed by heat. The enzyme did not appear to be identical with the acid phosphatase of milk (266). From experiments on the hydrolysis of phenylphosphate Mullen concluded that there might be about five times more alkaline than acid phosphatase in cow mammary tissue. Adrenalectomy and replacement therapy with cortical steroids had no marked effect on the acid phosphatase of rat mammary tissue. Moore & Nelson (268) have made preparations of the cyclophorase of the mammary tissue of lactating guinea-pigs. They appeared to differ in some respects from preparations of kidney and liver cyclophorase. The same authors (269) have studied the autoxidation which occurs in lactating mammary tissue of guinea-pigs, and have shown that it is not enzymic since it occurs in tissue preparations which have been autoclaved. Folley & Watson (270), in work on the arginase content of tissues, have found that adrenalectomy reduced the arginase activity of the mammary tissues of lactating rats and that implanted cortisone was more effective than 11-deoxycorticosterone in restoring the activity. This was true also of the liver, kidney and intestine.

Blood. Yakushev (271), in work involving 636 cows with widely varying milk yields, found that there was a close positive correlation between the yields and the maximum arterial blood pressures. Blood pressure increased with age and was affected also by exercise and season. Data on the chloride content of bovine blood plasma have been published by Lengemann, Aines & Smith (272). They found that the level of chloride averaged 360 mg./100 ml. and that it did not vary significantly with age or stage of lactation. The milk chloride, however, increased from 116 mg./100 ml. in early lactation to 190 mg./100 ml. towards the end. Some observations on the serum proteins of cattle have been made by Garner (273) and on the plasma proteins of sows during gestation and lactation by Friedell, Foster, Catron & Dieckmann (274). Work on the fat and sugar contents of arterial and venous blood of lactating cows has been published by Garkavi & Altman (275) and on carotene and vitamin A levels in the serum of cows near parturition by Goodwin & Wilson (276).

Water balance. Water-balance experiments have been made with twenty-three cows on four farms by Yakushev, Alikaev & Kozin (277). They calculated that on the average for each litre of milk produced 3–4½ l. of water were ingested. Of the total amount of water liberated by lactating cows about a third was excreted in the faeces, a quarter in the milk and a fifth in the urine, the remainder being lost from the lungs or through the skin. The proportions lost in those different ways would, of course, vary with the milk yield, climate and other conditions.

ANATOMICAL AND RELATED ASPECTS

In the past year a new edition of C. W. Turner's book on the anatomy of the mammary gland has been published (278). It contains nearly 400 pages and 205 illustrations and is excellently

produced. All who are interested in the anatomy, physiology, histology and surgery of the udder will find this publication of great value.

Matthews and Swett have continued their well-known work on the producing ability of cattle and their form or type. In a well-illustrated bulletin (Matthews, Swett & Fohrman (279)) they have discussed the weight and capacity of the udder in relation to producing ability, age and stage of lactation in experiments involving 473 cows. Udder weight and capacity increased with age up to 6 years, the increases being greatest between the first and second lactation. Highly significant correlations were found between producing ability and udder weight and capacity in both lactating and dry cows. They concluded that a large dry udder was as good an indication of producing ability as a large lactating udder. The average udder capacity of seventy-eight Holsteins with an average age of 6 years 2 months was 67·9 lb. compared with 55·0 lb. for eighty-six Jerseys with an average age of 7 years. Swett and Matthews have also studied udder weight and capacity for heifers of various ages (Matthews, Swett & Fohrman (280)). For fifty heifers between the ages of 3 and 30 months udder weight increased by about 0·59 lb. per month, and between 9 and 30 months udder capacity increased by about 0·44 lb. per month. Udder development in calves from 3 to 6 months old has been investigated by Brown (281). No consistent difference was found between the udder size of small calves and that of large calves at the various age levels. Large calves appeared to have larger udders in the 3- and 4-month age groups, but in the 5- and 6-month groups the udders of both large and small calves appeared to be similar in size. Brown concluded that mammary tissue tends to reach a certain size by 6 months of age independently of body growth.

A brief abstract (282) has been published on the relationship between mammary development and heart girth in 528 Holstein calves ranging from 11 to 25 weeks old. The correlation between the two was seldom significant, but full details have not yet been published. For dairy Shorthorn steers, however, Kohli, Cook & Dawson (283) concluded that heart girth was the best measurement for use in the selection of breeding animals where rate of growth and efficiency of food utilization were important. The relationship between live weight and heart girth for 214 Jersey cows and heifers in Denmark has been examined by Østergaard (284), who has recorded regression equations relating these two factors in animals of different age groups.

Body weights and sixteen different body measurements have been recorded and analysed by Schelper (285) for Spotted Mountain, German Brown and Hinterwald breeds. For all three breeds the height measurements at birth were found to be 55–57% of the maximum finally attained. Touchberry (286) has collected data for the study of genetic correlations between five body measurements and weight and production in Holstein cows. The data provided no evidence that size and type are genetically correlated in a positive way with production, but rather the reverse as far as size and milk production were concerned. This observation is at variance with earlier findings on the same question, and although the paper contains much of interest and importance to the geneticist the author himself seems a little doubtful about the interpretation of the data regarding this particular point. The relative merits of breeding for small or large animals with the object of producing more milk is being argued in a series of papers by de Groot (287, 288) and Van der Plank & Merkens in Holland (289, 290, 291). De Groot, writing in favour of small animals, points out that when cows are not kept longer than is general in the Netherlands, it is important to consider that the amount of fodder required for a live-weight increase of 100 kg. would be sufficient for the production of about 700 kg. milk. Van der Plank & Merkens point out, however, that with large animals of equivalent dairy merit the same amount of milk may be obtained with fewer cows and that this means less byre space, smaller costs for artificial insemination and reduced risk of disease.

The circulatory system of the cow's udder has been very thoroughly investigated by Swett & Matthews (292), who have produced an excellently illustrated bulletin on the subject. They point out that for long it has been the custom to regard the abundance of mammary veins on the surface of the udder, the size and length of the milk veins and the size of the 'milk wells' through which the milk veins pass as indications of milk-producing capacity, but from work involving

195 Holsteins and 89 Jerseys they obtained no evidence that these properties were of value for assessing milk-producing capacity. Removal of blood from the udder appeared to be limited to the external pudic veins and the subcutaneous abdominal veins (milk veins), and the external pudic veins were found to be so large and capacious that it seemed unlikely that the size of the milk veins could be of very great importance in relation to milk-producing capacity. For a cow which had produced about 15,650 lb. milk in 365 days, the circulatory system of the udder had a capacity of only about 4 lb. The blood vessels of the udder of the goat have been studied by Schauder (293).

The wall structure and closing mechanism of the bovine teat have been investigated by Pounden & Grossman (294), who rightly stress that few animal tissues are exposed to so much rigorous manipulation and risk of injury. They illustrate by diagrams and photographs that the wall of the teat has four layers, the skin and outer fibrous layer, the intermediate layer, the inner fibrous layer and the mucosa. Four structures appeared to be involved in the closure of the teat outlet, the muscle bundles, Fürstenberg's rosette, elastic fibres and desquamated epithelial cells.

The innervation of the udder of cows and sheep has been discussed by Peeters, Genie & Coussens (295).

SOME MISCELLANEOUS FACTORS AFFECTING MILK SECRETION

Climate. So great an increase has occurred in the last few years in publications on the effect of climate on the physiology and productivity of cattle that it is impossible to deal with them adequately in the present article. It may be helpful, however, if brief reference is made particularly to some of those that have a distinct bearing on lactation and milk composition. The subject as a whole was reviewed very fully in 1950 by Findlay (296).

Brody and his colleagues at Missouri have continued their extensive and detailed series of investigations on the environmental physiology of domestic animals. Descriptions of the work have been published in a number of bulletins in the past three years (297–308). They deal with the effect of ambient temperatures of 40 or 50° F. to 105° F. and of 50 to 0° F. on blood composition, heat production, cardiorespiratory activity, insensible weight loss, moisture vaporization, water and food consumption, and milk production. The cows and heifers used included Holsteins, Jerseys, Brown Swiss and Brahmans. It is not easy to generalize in a few sentences from such a wealth of data, but it can be concluded that milk yield and the efficiency of milk production were greatest at about 50° F. A critical high temperature at which rapid declines in production occurred was about 80° F. There appeared to be no critical temperature below 50° F., and for both Holsteins and Jerseys the declines which occurred in production at lower temperatures were much less marked than those which occurred at temperatures above 50° F. The decline in milk yield at higher temperatures was accompanied by an increase in fat percentage, particularly above 80° F. Holsteins were much more seriously affected by high temperatures than Jerseys (300). For Brahmans the critical temperature was 95° F., and the percentage decline in yield with rise in temperature was much less than in Jerseys in which it was less than in Holsteins. Increasing temperatures had no effect on the body weight of Brahmans but depressed the body weight of European cattle beginning at 80° F.; decreasing temperatures had no significant effect. Decline in food consumption began at 70–75° F. in European cattle and at 90–95° F. in Brahmans (303). In Brown Swiss cows (306) the critical temperature for rapid decline in milk production and body weight appeared to be 85° F. Regan (309), in California, has also found that at 80° F. in Holsteins and 85° F. in Guernseys and Jerseys the yield and non-fatty solids content of the milk markedly decline. The Jersey breed has been found to be readily adaptable to the climate of Puerto Rico (310), followed in order of adaptability by the Brown Swiss, Guernsey and Ayrshire breeds. Friesians were the least adaptable.

Details of work at Missouri on the effect of temperature from 5 to 105° F. on milk composition have been published by Cobble & Herman (311). Forty-three cows were used; they included

Jerseys, Holsteins, Brown Swiss and Brahmans. Above 80–90° F. the percentage of fat, chloride and total solids increased and that of lactose, total nitrogen and total non-fatty solids decreased. At low temperatures (4° F.) the percentage of fat, total solids, non-fatty solids and total nitrogen increased. Between 30 and 75° F. milk yield and composition in European breeds were not materially affected. In addition to the work at Missouri, studies on the effect of low temperatures (8 and 4° F.) on milk yield and composition have been made recently by workers in eastern Europe (312, 313, 314).

Other recent work on heat tolerance or acclimatization in cattle includes that by Badreldin, Oloufa, Asker & Ghany (315) in Egypt, Dobinson in Singapore (316) and Payne (317) in Fiji. Dobinson found that with Ayrshire, Holstein and Shorthorn cattle, when air movement is negligible, rectal temperature rises when the ambient temperature reaches 78° F. At that temperature the average rectal temperature was 101·8° F. after 1 hr. and 102·2° F. after 4 hr. Exposure for 2 hr. to a byre temperature of 94° F. caused the rectal temperature to rise to 104·5° F. When, however, heat loss was aided by sprinkling the cows with water every hour and by blast fans there was no appreciable rise in rectal temperature even at a byre temperature of 89° F. The value of water sprinkling to lessen heat load as measured by decrease in rectal temperature and respiration rate and an increase in grazing rate during the day has been shown also by Miller, Frye, Burch, Henderson & Rusoff (318) in Louisiana. In their work, however, the water-sprinkling treatment did not appear to result in increased milk yields. The authors point out that the increased grazing times during the day which the treatment brought about may have been counteracted by shorter periods at night or it may have been that the pasture was not of sufficiently good quality to stimulate increased milk yields. The effect of cooled drinking water in reducing heat load in Hereford and Brahman steers has been investigated by Ittner, Kelly & Guilbert (319), in California, who concluded that the use of cooled drinking water in their experiments 'added that little bit which tipped the balance toward the zone of thermal neutrality'. Ittner & Kelly (320) have also studied the relative merits of different types of erection for shading cattle, with and without spray cooling, in reducing the heat load which the cattle have to carry. The different types of shade are described in detail and illustrated by photographs. The importance of evolving new types of management which would be better suited to the tropics than standard methods used in temperate zones has been emphasized by Payne, Laing & Raivoka (321) in a brief discussion on grazing behaviour of dairy cattle in the tropics.

It is generally believed that in European breeds little heat, if any, is lost by true sweating as it is known in man, but that it is lost by evaporation from the lungs and diffusion of moisture through the skin. In the last few years Findlay and his colleagues (Findlay & Yang (322), Yang (323) and Goodall & Yang (324)) have made a very thorough histological study of bovine skin and have concluded that the sweat glands in cattle are apocrine and have a relatively poor blood supply and that they are unlikely to function as effectively in heat regulation as do sweat glands in man.

In a most interesting and critical article by Abrams (15) the possibility that sunlight plays a much more important part than is generally realized in influencing the reproductive and productive efficiency of cattle in temperate zones is very fully discussed. The article is too long to be adequately reviewed here, but it certainly deserves to be studied by all who are concerned with the environmental physiology of cattle.

J. A. B. SMITH

THE HANNAH DAIRY RESEARCH INSTITUTE
KIRKHILL, AYR

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